

Discrimination is not cricket

A meeting in Paris on 17 January will decide whether to persist with the ban on South Africans at this year's archaeology congress in Southampton. The organizers should cut their losses and cancel.

WHAT connection can there be between the exclusion of people from South Africa from the planned congress of the International Union of Prehistoric and Protohistoric Sciences (IUPPS) at Southampton later in the year and the demand, a week ago, by the government of Bangladesh for a public denunciation of apartheid from a group of not particularly distinguished English cricketers? Or, for that matter, with some of the reactions there have been to the continued scandalous detention of Dr Andrei Sakharov in Gor'kii? These questions are not riddles left over from the holiday season, but should directly concern the members of the executive committee of IUPPS, which will be meeting next week in Paris to decide whether the ban on South African participation at the congress should stand.

What can happen? The best outcome would be some ingenious means by which the congress could go ahead without the academic community inflicting permanent damage on itself and the rest of us, but the chances of that are slim. The next best course would be to abandon the congress, acknowledging that the organizers are in an impossible position from which there is no honourable escape. To continue with the arrangements that have been made, or further to fudge the issue, will be intolerable.

The circumstances leading to the mess in prospect have been fully catalogued (see *Nature* 318, 195; 1985). Briefly, responsibility for this year's congress was delegated by IUPPS to British archaeologists and anthropologists on the usual rota basis, but enthusiasm at Southampton for making the affair intellectually interesting and commercially successful must originally have seemed a plus. Planning fell to the British national committee, whose chairman, Professor John Evans, is also president of IUPPS, but most of the donkey work seems to have fallen to Professor Peter Ucko, secretary of the committee and also head of archaeology at the University of Southampton, the chief venue for the congress. By early last summer, the organizers had come under pressure to ban participants from South Africa. Pressure came from four different sources — students at Southampton (whose communal facilities were to provide space for a registration desk), the Association of University Teachers (AUT) which has a "national policy" that its members should not indulge in "academic relations" with South Africa, the British Anti-Apartheid Movement (which threatened to write to the embassies of anti-apartheid governments in London, and which may even have done so) and the Southampton City Council (which threatened to withdraw financial support and hospitality if South Africans were present at the congress). In September last year, the British committee capitulated, and South Africans were told they would not be eligible. There have been ructions ever since.

Cricket

Superficially, what happened last week in Bangladesh is not very different. As the English "B" team was gathering at London airport, the government of Bangladesh discovered that the names of three members of the party appear on a list, maintained by the United Nations in New York, of sportsmen who have played games in South Africa. The government demanded

that those concerned should denounce apartheid and promise never to go to South Africa again. The English cricket authorities, offended by the blackmail and fearful about the precedents that would be set, refused, and the tour of Bangladesh is cancelled. Cricket enthusiasts now fear that the games planned for West Indies in the summer of this year (to which the English would have to send an "AAA" team to stand any chance of winning) will similarly be wrecked.

Scholarship

If cricketers placed in an impossible position find it prudent to cancel planned events, why should not archaeologists do likewise? That is the obvious parallel, even if the reality is more complicated. Both cricket and scholarship have separately been offered as ingredients of the cultural cement that holds international society together, helping to provide hope that contemporary problems in international relations will one day be solved amicably, by reason. Although practitioners of both kinds are fond of saying that scholarship (or cricket) should be kept away from politics, their international doings unavoidably have political consequences, usually beneficial. The practical question, that cricketers and scholars must separately ask, is how best to conduct their international affairs so as not to compromise desirable objectives, among which the disappearance of the tragically wrongheaded policies of apartheid from South Africa must be one.

Circumstances matter. One reason why national sporting teams no longer go to South Africa is the fear that to do so would be read in South Africa as tacit approval for a distasteful system. Just how to balance that argument against the calculation that going will help to loosen the system is not easy, but the sportsmen are probably correct in the decision they have reached. In much the same spirit, it is unthinkable that one of the scientific unions would now arrange an international conference in Pretoria. By the same test, it would be wrong for a scholar to accept an invitation from the government of South Africa to embark on a lecture tour or for some other purpose that could be misrepresented. But it does not follow that South African scholars, as people, are no longer a part of the international community, or that they have nothing to contribute to its wellbeing. One of the ironies of the Southampton ban is that it will affect some of those who, by the accidents of palaeontology, have the most to contribute. To the extent that some of those affected are from the Universities of Witwatersrand and Cape Town, which have been bastions of liberality for the past three decades, the ban is also a cruel way of dealing with courageous people.

Mishandling

This is only part of the reason why IUPPS should next week overrule its British committee's decision on South Africa. No amount of agonizing by a group of archaeologists, anthropologists and ethnologists can make much difference to the course of events in South Africa, but the mishandling of the Southampton congress could do damage that will persist for longer than apartheid can. The invitations (in reality, they were advertisements) originally sent to South African scientists were addressed to



them as individuals, which was right and proper. The organizers have larded their ban with varying expressions of regret that the course they have felt compelled to follow will interfere with the free flow of ideas in science (and also infringe the statutes of IUPPS). There is wide sympathy for the position in which they find themselves, having to choose between the application of a principle they espouse and their understandable wish not to see months of careful planning wasted, perhaps at substantial cost. But there is no basis on which a group of scholars can exclude from an otherwise public meeting people who are qualified to attend, but whose government is socially unjust. To be the first in the field is a dubious distinction.

This is where the case of Dr Sakharov is relevant. Sakharov was an outspoken critic of the Soviet system a decade ago. He has not been charged with criminal activities, but merely sent to live in isolation from his friends and colleagues. Senior members of the Soviet academy say that Sakharov has been dealt with leniently, given the sharpness of his criticisms, but to their credit they have not expelled him from the academy, which would remove both his salary and his present degree of protection from administrative harassment. Even so, the case of Sakharov, like those of many other Soviet citizens who have suffered harsh administrative and legal penalties, has clouded scientific collaboration between Western and Soviet institutions. Yet at no stage has it been seriously suggested in the West that Soviet scientists as such are not welcome in the international community. If anything, the decay of formal collaboration has intensified the sense of loss that Soviet colleagues are absent from meetings at which they would naturally be found. Mercifully, but inconsistently, Soviet scientists have not been banned from Southampton. So why pick on South Africans?

The organizers say they have no choice, but they are wrong. Those who arrange meetings are always free to cancel them. Far better to stomach the embarrassment of wasted advertising literature than to be the first to introduce the principle of discrimination on the grounds of nationality into the conduct of intellectual affairs. Unless something can be done to make this congress respectable, it will probably be a tarnished affair, not merely on account of the notoriety it has attracted but because of the numbers of people who are threatening to stay away in sympathy with the handful of South Africans who will be excluded. The executive committee of IUPPS should bite the bullet and appeal to other scientific unions for help with the money problems left over.

Meanwhile, others must worry about the damage already done. The reputation of British academic institutions will have been harmed by this general advertisement of the power of campus demagogues, students and, lamentably, teachers. Does this mean that South Africans will be banned from all future international meetings in Britain? Does AUT's famous national policy deliberately invite this consequence, or is it merely a licence for local initiative by sporadic vigilantes? In their own interests, British academics should insist that the position of their representative body is clarified. AUT rightly spends most of its energy on academic pay and conditions of service, but its annual conferences may also adopt resolutions on more general matters. AUT's policy on South Africa is badly thought out.

More generally, there are international forces to be reckoned with. Although the constitution of IUPPS itself is explicitly non-discriminatory, the union is affiliated to UNESCO, which adopted a policy of deliberate discrimination against South Africans four years ago. Does that make sense? There is a more sinister threat in the statement by one of the Southampton organizers that it is no longer possible to organize an international conference to which "third world" scientists are invited without discriminating against South Africans. That cannot be the whole truth (witness the attendance of several South African astronomers at last December's meeting of the International Astronomical Union in Delhi), but even if it were a part of the truth, it would be a bad business for us all. □

Chopper politics

The British government seems bent on making a muddle of its policy on helicopter manufacture.

WESTLAND, a small manufacturer of helicopters based in south-west England, has become the centre of a bewildering row between British cabinet ministers for all the wrong reasons, which between them should provide a salutary warning for high-technology industries dependent on defence departments for their survival. The troubles stem from the nature of the helicopter as a means of transport. Although it was once commonly supposed that such machines had a bright future as means of almost personal transport, experience has shown them to be an expensive means of getting about, uncompetitive with fixed-wing aircraft over distances much greater than 100 kilometres and with surface transport over shorter distances. The only civil markets for the machines that matter are tiny, and are confined to circumstances where other means of transport do not exist (as in the servicing of oil-rigs at sea) or where a few minutes saved on a journey time may command a high price. (There is still a market for helicopter journeys between New York's principal international airport and Manhattan.) Otherwise, the helicopter has become almost exclusively a military vehicle, a means of transport and a battle-station.

Westland has known this for some time. For the past year, it has been pleading with the British Ministry of Defence under Mr Michael Heseltine for extra projects on which to work, and has been refused. So, under pressure from its bankers, Westland has been seeking larger companies willing to mount what is called in the trade a "rescue". The US corporation United Technologies, which owns Sikorski, another helicopter manufacturer, in conjunction with Italian Fiat, was the first in the field. Mr Heseltine has since organized a bid from a European consortium of aircraft manufacturers which differs chiefly in the amount of employment it offers the labour force at Westland. Members of the British cabinet have fallen out among themselves because some (including the Prime Minister) rightly say that the company should decide on commercial ground which bid to accept, while Mr Heseltine (who is not alone) threatens that Westland will not again win European orders for helicopters unless it accepts the bid he has organized (which it is within the power of European governments to ensure).

The piquancy of the resulting row derives from its bearing on British domestic politics. What the participants have forgotten is that Westland's problems of a year ago were a sign that there were then more helicopter manufacturers than the world's defence ministries could employ. Nothing much seems to have changed since then. And nothing has happened to suggest that Westland's technical dependence on other manufacturers (Sikorski in particular) will have been replaced by technical adventurousness in an admittedly difficult field. Mr Heseltine's European bid smells too much of an attempt to keep in being a company that is otherwise not viable by means of a redistribution of helicopter work among European governments.

The lesson to be learned from this should in reality have been learned a long time ago. The prizes now go to those who make technically advanced products of high quality. In civilian markets, to be second or third best is to invite disaster, but in the military field, chauvinism can keep projects afloat for longer than logic would allow. The fate of Westland has become an issue largely because the British government has resolved to let economic logic apply more rigorously than will other European governments. Mr Heseltine's laudable ambition that European defence forces should have access to the best equipment could just as well be satisfied by letting Westland go to the highest bidder and, quite separately, planning with European governments a strategy for buying (not necessarily making) the next generation of military helicopters. That way, he would discover, British taxpayers would find themselves paying less. □

Japanese technology

First sale overseas of military equipment

Tokyo

Just before Japan's companies closed for the new year holiday, a small but potentially momentous technology deal was completed between the United States and Japan. The price of the deal is secret, but even if rumours that the US side moaned at having to shell out \$40 million are correct, it will hardly add much to a mountainous trade deficit. The significance of the deal is that it is the first sale of military equipment by Japan to a foreign country since the Korean war.

The equipment sold to the United States consisted of anti-aircraft homing devices that, in contrast to the usual heat-seeking technology, guide missiles to their targets through visual tracking. The equipment employs charge-coupled devices similar to those used in the "seeing eye" sensors of autofocus video cameras. Japan has long been a leader in such research. The homing device was developed by the Technical Research and Development Institute, the government research body responsible for the development of weaponry for Japan's Self Defense Forces.

"Self-defence forces" is what they have to be called because Article 9 of the Japanese constitution specifically states that "land, sea and air forces as well as other war potential, will never be maintained. The right of belligerency of the state will not be recognised." Even self-defence forces are of doubtful constitutionality and must be as non-belligerent as possible. Defence is an extremely sensitive issue and a subject of continuous national controversy. The present government, which favours expansion, is hamstrung by the actions of Prime Minister Miki's government ten years ago. Back in 1976, it was decided that defence spending should be kept within 1 per cent of gross national product (GNP). (In contrast, the United Kingdom spends 5.6 per cent of GNP, a difference that has helped the development of Japan's non-military technology.)

This guideline, for it is nothing more, has now become an impenetrable barrier. Over the past year, Prime Minister Yasuhiro Nakasone has tried a hundred different roundabout ways of saying the limit would have to go, but without success. This year's defence budget is set at 0.993 per cent of GNP, provided the economy grows as well as is hoped. If UK accounting methods were used, Japan would already be spending 1.6 per cent of GNP. Over the years, some defence-related items have vanished from the defence

budget; there was even once a suggestion that the troops' food was not strictly a defence item and could be taken out of the budget.



Ten years ago, it was also decided that export of any kind of weapon or weapons component to any country, friendly or unfriendly, would be prohibited. This ruling has been enforced: when a company exported a metal component for a hand grenade trigger, the police took action. But that has not altogether prevented some bending of the rules. Japan, after all, manufactured the floating dock moored in Vladivostok for the massive Soviet aircraft carrier *Minsk*, on the grounds it was

not necessarily for military use. More recently, under pressure from Washington, the United States alone has been excepted from restrictions. In November last year, the first Joint Military Technology Commission meeting was held to promote the transfer of military technology to the United States. Interest was apparently shown in high-speed gallium arsenide microchips and optical fibre communications equipment. Now the first technology sale has gone ahead.

The next big question for the Japanese government will be whether or not to participate in the US Strategic Defense Initiative (SDI). The decision by the United Kingdom to join the project is felt to have put the heat on Japan — it will have to say yes or no, rather than just trying to pretend that it had never been asked. Prime Minister Nakasone is known to be in favour of the project and the Foreign Minister has recently stated that the United States cannot be kept waiting too long for a decision. Both have repeatedly stressed that SDI is "defensive" and "non-nuclear". Government experts say participation would not be unconstitutional.

Business circles, on the other hand, cannot make up their minds whether they might be really missing out on some totally new technology if they do not join the project. But when all is said and done, it will still require an enormous push for Japan to join SDI. There is still only one issue that really unites the great mass of the people against the government that has been in power for 30 years — any hint of a move back towards militarism.

Alun Anderson

US science adviser

McTague fills Keyworth's shoes

Washington

JOHN McTague has been named acting director of the White House Office of Science and Technology Policy (OSTP), replacing George Keyworth II who resigned at the end of last year. McTague has been with OSTP since 1983 as deputy director.

McTague's temporary appointment has surprised many, as it was widely expected that Erich Bloch, director of the National Science Foundation, would be given the additional title of acting director of OSTP until a permanent replacement could be chosen. Indeed, that had been Keyworth's recommendation, but according to OSTP spokesman Maurice Roesch, acting director for defence programmes, "the idea was considered and rejected". McTague's interim appointment was a "collective decision" of the White House personnel office and OSTP.

McTague is a physical chemist. He was at the Institute of Geophysics and Planetary Physics at the University of California at Los Angeles from 1970 to 1982, after

which he served as chairman of the National Synchrotron Light Source Department at Brookhaven National Laboratory until he joined OSTP.

McTague has enjoyed a good working relationship with Congress. Ezra Heitowitz, staff director for the House of Representatives subcommittee on science, research and technology, describes him as "helpful and forthcoming with staff members".

A public supporter of the Strategic Defense Initiative (SDI) proposed by President Reagan, McTague is nevertheless unlikely to be as actively involved in promoting SDI as his predecessor.

The failure to name a permanent replacement for Keyworth will keep alive rumours about the possible demise of OSTP. But Roesch insists that the search for a permanent director is being carried out "as rapidly and expeditiously as possible", although he could suggest no timetable for when that permanent appointment could be expected. **Joseph Palca**

British computing

Cooperative research spends up

THE Alvey Programme, Britain's answer to the Japanese fifth-generation computing programme and the US integrated circuit programme, has now committed almost all of its £350 million budget. The money is being spent on "cooperative pre-competitive research in information technology" through the Department of Trade and Industry (DTI), the Ministry of Defence (MoD) and the Science and Engineering Research Council (SERC).

Alvey's director, Brian Oakley, says that a "very useful programme" has been mounted in the past two years, although he is not impressed by some of the government's financial restrictions; for example, no extra posts were provided for the programme, meaning that it is run on "the proverbial shoestring". Money for Alvey comes from funds that each department had already earmarked for other uses.

Alvey projects fall into five main categories: intelligent knowledge-based systems (23 approved projects); man-machine interface; very-large-scale in-

tegration (VLSI, exclusively focusing on silicon technology) and computer-aided design; software engineering (16 approved projects); and computer architecture. Of 550 proposals submitted to Alvey, 102 have been approved, 6 per cent of which are long-range speculative projects in universities. Each approved project includes an average of two or three industrial teams and one or two academic teams, ranging from two to eight partners per project.

Oakley admits that some mistakes have been made. One example is the Alvey requirement that, once a project has been technically approved, a collaborative agreement covering industrial property rights is required before money is paid. But the lack of advice or guidelines from Alvey has resulted in delays while arguments between partners were sorted out. "Quite inexcusable bureaucracy" resulted from the decision to divide up administrative duties — VLSI contracts to MoD and academic grants to SERC. The intention

was to minimize the central administration team and to involve all participants in administrative decisions, but in fact bureaucratic differences and delays resulted. Oakley recommends that a non-governmental body should administer future collaborative schemes. Ten per cent of approved Alvey projects are also delayed by SERC's cash-flow problems, but Oakley foresees an end to these problems early in 1986.

Despite the problems, Alvey's annual report* provides evidence that a strong technology base is being provided in Britain by a directed collaboration between government, industry and academic research institutions. Alvey is halfway through its five-year life; the future of information technology research after this time is uncertain. Should Britain maintain its own attempt to compete with the United States and Japan or combine forces formally with ESPRIT, the European Strategic Programme of Research and Development in Information Technology?

Oakley is in no doubt that there is a strong need for both a national and an international programme. Geoffrey Pattie, minister for industry and information technology, hopes that a working group will be set up, with industry playing a "leading role" in deciding what is to be done.

Maxine Clarke

* Alvey Programme annual report and poster supplement 1985. Price £10 each from Institute of Electrical Engineers, PO Box 8, Southgate House, Stevenage, Herts SG1 1HQ, UK.

Siberian river scheme

Russian party demands caution

A GROUP of eminent Russian writers has called for the cancellation of the proposed southward diversion of the Siberian rivers. In a letter published last week by the daily *Sovetskaya Rossiya*, they say that the scheme "threatens serious consequences not only for future generations but also for those alive today". They condemn the project as an interference in "natural conditions that have built up over millions of years" and claim that the ecological, economic and social consequences have been insufficiently considered. Although opposition to the project has been voiced by Soviet environmentalists since the idea was revived in the mid-1970s, the writers' letter is unusually outspoken, attacking as "premature" the inclusion of the diversion scheme in the "Draft Guidelines" of the new five-year plan. Although the guidelines will formally remain open for public comment until the Party Congress next month, this remark questions the professional expertise of the high-level team responsible for this part of the plan, suggesting not so much a general relaxation of press censorship as the seriousness of concern over the diversion scheme. The appearance of such a letter, signed not by professional environmentalists, but by literary figures who in the West might be expected to show "green" tendencies, is itself significant.

The group includes Dmitrii Likhachev, perhaps the most eminent Soviet literary critic on the mediaeval period, and Leonid

Leonov, whose literary career dates back to the early days of the Soviet Union. For several of them, this is not their first expression of hostility to the scheme — Vasilii Belov, one of the best known of the group, actually published a letter attacking the scheme in the Paris emigré newspaper *Russkaya Mysl'*, but it is their first public statement on the matter in the Soviet press.

Although they span three literary generations, the writers are all strongly committed to the traditions of old Russia. Some consider that the group constitutes a potentially significant political force, dubbed it the "Russian Party".

The fortunes of the group during the past few years have not been unclouded — during the June 1983 Plenum of the Central Committee, their views were attacked by Konstantin Chernenko, at the time propaganda secretary under General Secretary Yuri Andropov. Nevertheless, they have been largely responsible for the government's pledge that "monuments of Russian antiquity" should not be damaged. Such an assurance is significant in the context of Soviet hydroengineering, which before the Second World War submerged, among other places, the Dnieper rapids (analogous, for a Ukrainian, to drowning Runnymede or Harper's Ferry), and also the important archaeological site of Ithil, capital of the Khazars, the one gentile nation in the past 2,000 years to have converted to Judaism.

Vera Rich

Soviets ask for space

ACADEMICIAN Anatolii Aleksandrov, president of the Soviet Academy of Sciences, has called for a world space organization for international cooperation in the peaceful study and use of space. Such an organization, he said in an interview in *Pravda*, would ensure access for all states "on the basis of mutual advantage" to the data from meteorological, Earthscan and navigational satellites, and would be particularly beneficial to developing countries that cannot afford their own space programme. It would, furthermore, provide a framework for major cooperation in space research, and would also, by its very existence, "epitomize peaceful intentions in space".

The proposed organization, Aleksandrov suggested, would coordinate the activities of existing international bodies. He spoke glowingly of Soviet participation in the international KOSPAS-SARSAT experimental sea-rescue service jointly with the United States, Canada, France and Norway, and in the 10-nation Venus-Halley's comet programme.

Not all space cooperation, however, runs auspiciously for the Soviet Union. A few weeks ago, there were reports of a major rebuff by the council of Inmarsat,

Synthetic fuels

US Synfuels narrowly bites dust

Washington

THE Synthetic Fuels ("Synfuels") Corporation (SFC), willed into existence by Congress in 1980 with an annual spending power of \$20,000 million, was legislated out of existence just before Christmas. But the controversial corporation's end was only narrowly prevented from turning into a \$600 million spending spree.

SFC was formed when oil and natural gas prices were expected to continue to rise sharply, making the development of other sources of such fuels economically realistic. SFC's job was to support companies attempting to develop these alternatives. But reality turned out differently from expectations. Oil and natural gas prices did not rise precipitously, while large-scale projects for producing fuel from oil shale and natural gas from coal encountered technical problems for which expensive solutions were required.

In more than five years, only one of SFC's projects actually reached the point of producing energy on a commercially usable scale — the Cool Water coal gasification plant in California, which feeds into a combined cycle generator to produce electricity.

As its future came to seem less and less bright, members of Congress began to attack SFC as a rogue elephant, claiming

that it was poorly managed and that its goals had in any case receded out of reach. Congress has also known that the Reagan administration has been sceptical of SFC since its inception towards the end of the Carter presidency. After fierce negotiations last year, by the end of the summer it was clear that SFC's days were numbered.

That prospect spelt disaster for two of SFC's projects, the Union shale plant at Parachute Creek in Colorado and the Seep Ridge oil shale project in Utah, both of which are still in need of SFC backing if they are ever to be viable. This dire prospect seems to have provoked the high drama in the days and hours before and after the congressional vote on 16 December.

Two interested senators, Jake Garn (Republican, Utah) and William Armstrong (Republican, Colorado) appear to have taken the initiative in asking the White House to allow the SFC board to meet and to provide the funds for the projects at risk before Congress had finally voted the corporation out of existence. If that had happened, the government would still have been liable for the com-

mitments, in excess of \$600 million.

Reports of what was afoot reached Capitol Hill together with the intelligence that the President's chief of staff, Donald T. Regan, has blessed the plan for a final meeting of the board, and even that the president might delay signing an abolition bill so as to give SFC a last chance to act.

The board of governors of SFC seems to have been in a difficult position. On the one hand, there were signals from the White House apparently authorizing new spending, but, on the other, clear indications that Congress wanted to call a halt to synthetic fuels development. In the end, the board decided not to act without written instructions. There appear to have followed several heated telephone conversations between members of the Congress and the White House, at the end of which the board was instructed to spend no more public money.

Ironically, the measure abolishing SFC was appended to a larger financial bill which was, in the end, defeated in the vote on 16 December. For a while, SFC was back in limbo, but supporters of SFC were unable to engineer a last-minute reprieve for the corporation, which was finally abolished when the larger financial measure was passed on 19 December. **Joseph Palca**

British biotechnology

Celltech within sight of profit

CELLTECH, Britain's budding biotechnology corporation, greeted the new year with an annual report showing a 27 per cent decrease in losses and a forecast of profitability by 1987. In the year ending 30 September 1985, Celltech's £3.8 million turnover more than doubled the previous year's. Chief executive Gerard Fairtlough reckons his company is the world leader in the bulk production of monoclonal antibodies, and is looking to Japanese and US markets, which account for about 85 per cent of Celltech's customers.

Although Celltech's cash balance fell from £9.8 million to £5.7 million during the year, Fairtlough says there is no present need to increase the company's equity base. Rather, Celltech is likely to rely on joint ventures of the kind that established Boots-Celltech Ltd in 1983 and Apcl Ltd in 1984. At its outset in 1980, Celltech gained impetus from an exclusive and widely-criticized agreement with the Medical Research Council (MRC) for first rights of refusal on all pertinent research. Although the five-year agreement was significantly qualified in 1983, Celltech's finance director Hugh Perrott says that the group still enjoys close collaboration with the council's laboratories.

Celltech's crowning accomplishment so far has been the development of a 1,000-litre fermentation tank, which churns out kilogram quantities of the antibodies that

the US company Ortho Diagnostic Systems Inc. will market worldwide as blood group reagents.

Monoclonal antibody production occupies most of Celltech's culture products division, which also supplies antibodies for the purification of interferon and interleukin-2. Last year Celltech spent £2.4 million on expanding these facilities.

The health care division has agreed with the Japanese pharmaceutical firm Sankyo to develop calcitonin, potentially a treatment for osteoporosis and Paget's disease, and tissue plasminogen activator, which dissolves blood clots, for commercial availability. In the United States, Serono Laboratories Inc. has contracted Celltech to produce human growth hormone in mammalian cells and is already conducting clinical trials.

Fairtlough says he has suffered only one disappointment in his company's short history: chymosin, Celltech's first recombinant DNA product, had to be shelved because of patent difficulties and an overestimation of the demand. Fairtlough contends that the dwindling (50 to 15 per cent) shareholdings of the British Technology Group (BTG), which provided the financial catalysis for Celltech's beginnings, does not reflect a lack of faith on BTG's part. He says that Celltech's shareholders are as satisfied as its customers.

Karen Wright

collaboration

the international programme for establishing a system of geostationary navigational satellites. According to the Soviet side, the list of rocket launchers initially accepted by Inmarsat includes the Soviet *Proton* rocket, which, the Soviet participants claim, is cheap and meets the programme's requirements. The United States has now opposed the use of the *Proton*, on the grounds that a launch from the Baikonur site would give the Soviet team the chance to acquaint themselves with the US equipment aboard the satellites, and remains unconvinced by Soviet assurances that the delivery of the rocket to the launch-site, preparations for launching and the launch itself would be carried out in conditions that would exclude any "information leaks" about the on-board equipment.

The dispute over the abandonment of the *Proton*, which the Soviet side claims will mean a loss to Inmarsat of several million US dollars, was not mentioned by Aleksandrov. His vigorous urging of the benefits of pooling knowhow and economic assets in global space organization acquires, in the light of the Inmarsat disagreement, an additional topical note.

Vera Rich

Bell Labs

From monopoly to competition

Murray Hill, New Jersey

REPORTS of the death of research at AT&T Bell Laboratories have been exaggerated. Since AT&T spun off seven regional telephone operating companies representing two-thirds of its assets at the start of 1984 in settlement of an anti-trust case, Bell Laboratories have indeed changed. But fears that the new slimmed-down AT&T would be unable or unwilling to support basic research in the style of the past have not materialized.

For 60 years, Bell Labs have been the world's foremost electronic research laboratories. Ian Ross, Bell Labs' president, argues that with a budget of \$2,000 million and a staff of 19,000, the laboratories are of the scale expected of a company the size of AT&T (operating revenues in 1984: \$33,000 million), and that basic research is now more important to AT&T than ever. What has changed is that the development staff, representing (as it did before 1984) some 90 per cent of the total, is now working more efficiently and with increased urgency to exploit usable research results to develop what he calls "killer technologies": technologies that will, like the transistor, lead to the demise of what went before. Basic research, he insists, remains untouched.

The corporate restructuring has, however, been far from painless. In the year following divestiture, Bell Labs lost 4,000 of their then 24,000 staff to AT&T information systems, which manufactures computers, and a further 4,000 to Bell Communications Research, the company set up to conduct research for the newly liberated local telephone companies. Some of the lost ground has since been regained, and Ross has used the opportunity to push Bell Labs in what he sees as the direction of AT&T's future: "we are", he says, "running like hell to change the technology mix".

The 1982 settlement that led to the break-up of AT&T also affirmed an earlier ruling of the Federal Communications Commission that allowed it to enter unregulated markets in information-handling. Ross's greatest hope for Bell Labs is that they will achieve a "fundamental solution to the problem of how to handle software", in which progress worldwide has been less spectacular than in hardware. Almost half of Bell Labs' employees are now producing software as their end-product, and Ross expects to see the proportion rise.

The Strategic Defense Initiative (SDI) is one area that would benefit from fundamental software advances and Ross is making bids on SDI contracts. Solid-state physics and chemistry, once at the forefront of Bell Labs' research, are by com-

parison now taking a back seat, while only a handful of researchers in economics and psychology remain to keep AT&T with a "window" in that field.

Not that Ross has abandoned physics; there are, he says, still two orders of magnitude of improvement in the circuit density of silicon integrated circuits to be achieved before fundamental constraints are reached, and three orders of magnitude of improvement in optical data transmission rates. Ross clearly intends that Bell Labs should maintain their pre-eminence in this area.

But for all Ross's enthusiasm, it is plain that the uncertainty of 1984 did cause a crisis of morale among Bell Labs' researchers. The losses to Bell Communications Research (Bellcore) and AT&T Information Systems were keenly felt; in Bell Labs' photonics research laboratory, for example, Bell Labs researchers work alongside Bellcore staff but are forbidden to share their research results. That irritation should soon be removed, when the Bellcore workers move into a new building now under construction. But AT&T's uncertain finances, which have caused large lay-offs in some manufacturing divisions, have led to what Ross calls a "salary structure freeze" across the company, including Bell Labs, meaning that salary increases are not awarded automatically. Ross says the "expectation" is that salaries "will be unfrozen" before long.

Many researchers, however, go to work at Bell Labs in part because of their reputation for having an easy collegiate atmosphere with the freedom to pursue academic interests. The introduction of symbols of corporate formality such as security checks for visitors and identification badges for staff therefore caused much resentment, and some took the changes as a cue to leave, most to go to universities.

One who did leave Bell Labs, Terry Miller, went to become professor of chemistry at Ohio State University; he says his decision was the result of a tempting offer and uncertainty about the climate for research at Bell Labs, but he remains in contact with former colleagues and does not now hesitate to recommend a research career there to inquiring students.

Not all divisions seem to have experienced attrition. Kumar Patel, executive director of research for physics and academic affairs, noted no change, but said the changed circumstances of AT&T meant that he had to "do more explaining" to hire promising researchers. But Patel's division has not been untouched by the changes to AT&T. Physicists in the division have shown the possibility of building a positron microscope, which

would be an analytical tool with unique properties. "In the old environment, we would have done nothing about it", said Patel. Now, development staff are watching progress closely, and within two years a business decision will be taken on whether the positron microscope should be the target of a major Bell Labs development effort.

Arno Penzias, Bell Labs' vice president for research, is clearly stung by suggestions that AT&T's new competitive spirit will mean an end to the sort of unfettered basic research that led, for example, to his Nobel-prize-winning discovery of the cosmic microwave background radiation. Penzias defiantly points out that astrophysicists still work at Bell Labs, the justification being that the tools of their research may have applications to AT&T's technological base. High-energy physicists, by the same logic, are not employed. And Penzias insists that basic research staff, who now number about 1,000 ("crudely where it's been for a long time") are still encouraged to pursue long-term projects that might bring benefits in five to 20 years rather than more short-term gains. Researchers realize that they are protected as far as possible from the effects of fluctuating budgets, and most feel the worst of the crisis is over.

Penzias says that long-term strategic research at Bell Labs has always been important because, even before divestiture and despite the appearance of monopoly, AT&T always faced pressure to be competitive on prices. (Penzias cites AT&T's response to the establishment of COMSAT, which was to develop optical transmissions.) The change represented by divestiture for Bell Labs is merely in the way it benefits from successful research; before, it was mainly through cross-licensing agreements (the old AT&T was forbidden from benefiting directly from spin-off from its research), while now "one can envisage a script where AT&T cannot afford to pay its dividend". The effect is that research has "moved from being an important part of AT&T's prospects to a vital part".

Laboratory managers have been told to work harder to identify promising research breakthroughs at an early stage in order to alert promising development workers; while the development division has been totally reorganized into product line groupings that correspond to the activities of AT&T's other subsidiaries. Before divestiture, a researcher who found a result of possible interest would "write a memo, throw it over a wall and wait for someone to answer", according to David Lang, head of semiconductor and electronics research: "Now they answer the telephones".

Penzias says the salary scheme for basic researchers at Bell Labs continues to encourage excellence in research, which is

assessed and closely monitored by laboratory managers. (Efforts by researchers to ensure that their ideas are taken up by development staff are, however, now rewarded by salary bonuses, an innovation to reflect the changing times.) And Penzias believes in allowing researchers total freedom of inquiry. Mitchell Marcus, a linguistics and artificial intelligence researcher, agrees that he is given "more freedom than in a university". Alan Huang, who heads what may be the only corporate optical computing research department in the world, says he was "startled" shortly after divestiture to be told to take more risks in his research.

Huang's futuristic theorems on pattern recognition algorithms that might one day form the basic logical operations in optical computers are clearly exceptional, but his upbeat assessment of the post-divestiture working environment at Bell Labs is shared by many others. However, there is a niggle: Bell Labs have made a decision to be more careful about the information they will allow researchers to publish.

The irritation arises because recognition by peers is an important form of feedback, not entirely compensated for by the knowledge that permission to publish is being denied because of the possible importance to AT&T of the results being reported. Ross says he favours publishing more, but with "more on what we've done and less on how we did it". Fewer than one per cent of the 4,000 papers submitted for publication clearance each year are turned back for revision, but it seems to be accepted that irritation over this has increased: Ross says he is watching the subject "closely". Penzias cites the case of Narendra Karmarkar's celebrated algorithm in linear programming, now being used by AT&T for designing Pacific basin communication networks, as an example of the successful and judicious application of the policy: although Karmarkar's particular implementation of the algorithm remains proprietary, enough details were released for publication for Karmarkar to receive public recognition and win academic prizes.

Ross opposed divestiture at the time the question was being examined in court, and says he still believes the decision was a mistake. But it has allowed — or obliged — Bell Labs to be more responsive to technical developments. Ross complains that Bell Labs' most famous invention, the transistor, was brought into every home in transistor radios by other companies while AT&T spent 20 years in developing a version reliable enough for use in its only permitted application: telephone switching equipment. When the next "transistor" is invented, Ross says, "we can take it wherever we want". And, perhaps with good reason, he seems to have no doubt that there will be a next time.

Tim Beardsley

Israeli–Egyptian cooperation

Minor relic of Camp David

Rehovot

THE grandiose dreams of large-scale scientific cooperation between Egypt and Israel that flowered in the wake of Anwar Sadat's visit to Jerusalem in November 1977 and the peace treaty of March 1979 have not materialized. Nevertheless, despite intervening political storms, joint research projects in agriculture, mariculture and medicine have been quietly taking place.

As with the Camp David negotiations, US go-betweens have played an important role; the trilateral Cooperative Arid Lands Agriculture Research Programme (CALAR) was set up by researchers from Egypt, Israel and the United States at the University of San Diego, California, in June 1981, with financial support from the US Agency for International Development.

Although research programmes are developed and carried out independently in each participating country to suit specific conditions and needs, experience has shown that lessons learned in one are generally applicable in the other. This is certainly true of the exploitation for agricultural purposes of the abundant quantities of saline water in both Israel and Egypt.

One of the participating scientists, Professor Jehoshua Rudich of the Hebrew University's Faculty of Agriculture in Rehovot, points out that the Israeli practice until now has been to dilute saline water found in its desert regions (normally some five times as salty as ordinary irrigation water) with fresh water until it is only twice as saline. It is then used to irrigate salt-tolerant crops, notably cotton and melons.

More recently, within the framework of the CALAR programme, undiluted brackish water has been used to grow tomatoes (following the use of small quantities of sweet water to reach the flowering stage). The yields were slightly below normal, but the tomatoes were of high quality colour, acidity and flavour.

In parallel work in Egypt, Dr Adel S. El-Beltagy and his colleagues have shown, according to a recent CALAR report, that the local Edkawy variety of tomato is highly suitable for cultivation with saline water. They are, at the same time, producing water of various degrees of salinity and testing it with different methods of irrigation (drip, furrow), as well as studying how yields are affected by such factors as planting distances, windbreaks and fertilizers.

Another CALAR project in both countries involves the development of drought-resistant forage shrubs to feed sheep and goats. While the exploitation of brackish

water is naturally emphasized, Dr Dov Pasternak of Israel's Ben-Gurion University, has set his sights on using water taken directly from the Mediterranean. His team has achieved encouraging results with approximately 50 plant species, most of them from the parched interior of Australia.

The Egyptians, meanwhile, have achieved significant progress in cross-breeding the Barki goat, a hardy native of their desert regions but a low producer of milk, with the high-producing Damascus goat.

A third sphere under investigation is the cultivation of crops that can be used as industrial raw materials. Successful results have been achieved in the deserts of both countries with jojoba, guayule and buffalo gourd.

Although political factors have kept cooperation out of the headlines, it has been reflected in more than a dozen joint Israeli-Egyptian presentations at international gatherings as well as joint papers in international scientific journals, mostly on the endemic parasitic afflictions leishmaniasis and malaria.

Medical cooperation began, however, with the mosquito-borne viral disease called Rift Valley fever. Ordinarily confined to central and southern Africa, it has killed 598 people and thousands of animals in Egypt since 1978. Israeli experts, fearing a further spread of the disease, readily agreed to a suggestion by the United States of discussions with their Egyptian counterparts.

Israel's main contribution has been made by a team led by Professor Rachel Galun of the Hebrew University's Kuvim Centre for the Study of Infectious and Tropical Diseases, which carried out studies on the behaviour of the mosquitoes that transmit the virus to cattle, sheep and man. Her findings were almost certainly of use in helping Dr Sherif El-Said of Ain Shams University and his colleagues in their successful efforts first to monitor and then to stamp out Rift Valley fever in Egypt.

Hebrew University researchers also worked together with Egyptian doctors and scientists after an outbreak of visceral leishmaniasis in Egypt. On that occasion, efforts in Jerusalem centred on the detection of antileishmanial antibodies by use of a new method that was developed there.

"Like all good cooperation", Professor Galun explains, "that between the Egyptians and ourselves has been based on mutual self-interest. For we both realize that infectious diseases and the vectors that carry them do not respect national boundaries."

Nechemia Meyers

Comecon technology

Plans laid for collaboration

THE Soviet bloc seems on the brink of novel schemes for technical collaboration. The Comecon economic summit in Moscow last month outlined five key areas of cooperation in its "Comprehensive programme" for collaboration among member countries "up to the year 2000". The five priorities, electronics, automation, nuclear power, new materials and biotechnology, hold no surprises — they were already earmarked for "integrated" cooperation at a previous summit in June 1984. More interesting, however, are the measures proposed for implementing cooperation, and the suggestion that the new programme could be the basis for cooperation outside Comecon itself.

The programme notes that the member countries have decided to translate the priorities "immediately" into specific agreements and contracts, embracing all stages of the "science/technology/production/market" process. The programme provides for direct relationships between scientific organizations and production enterprises in the member countries. Such relationships have until now usually been channelled through high-level organizations situated in the capitals of the countries concerned. This organizational development may fit in with the interest expressed by some Comecon countries in technical collaboration with West European governments. Direct relations of the kind now proposed would thus facilitate possible cooperation between the "comprehensive programme" and the West European Eureka programme, in which companies rather than governments will be the principal actors.

Comecon interest in Eureka has not been great, and the Czechoslovak party daily *Rude Pravo* has been downright hostile, alleging that Eureka and the US Strategic Defense Initiative (SDI) are closely linked, and that many companies likely to be involved in Eureka have already been contacted by the Pentagon, and will work simultaneously for both projects.

The one Comecon country to show real interest in a possible link-up with Eureka is East Germany, an interest that dates back to the visit of French Prime Minister Laurent Fabius to East Germany in June 1985. Perhaps significantly, the main Western interest in such cooperation seems to be coming from West Germany. Last month, Rudi Arndt, chairman of the socialist group in the European Parliament and himself a member of the West German Socialist Party (SPD), told a Moscow press conference that the delegation he had brought to Moscow might "smoothe the way for a formal agreement between the EEC and Comecon", and

noted that the prospects for cooperation between the "comprehensive programme" and Eureka were "good".

The Soviet response to Eureka has so far been noncommittal. A Moscow television press conference after the Comecon summit noted that "there are a number of spheres of research and development of new technologies which are to be found in our programme and in the Eureka programme", but pointed out that the decisions leading to the Comecon programme were taken in June 1984, "at a time when no one had yet heard of Eureka".

West German space

Ministers at odds about Hermes

Bonn

THE federal ministers for research and foreign affairs are sharply in conflict over the proposal that West Germany should participate in the French plan to develop a space shuttle called Hermes. Heinz Riesenhuber, the Christian Democrat (CDU) Minister for Research and Technology, has set his face against collaboration in the project, saying that this could not be justified within the framework of his ministry's strategy for research and development. But the Free Democrat (FDP) foreign minister, Hans-Dietrich Genscher, is keen that West Germany should collaborate in the French project for the sake of Franco-German relations.

Riesenhuber's objections to collaboration have their root in budgetary problems. The minister says that German collaboration could not be financed out of the research budget as it stands, and that if West Germany is to take part in the Hermes project, the federal government will have to allocate extra funds for the work. This position is consistent with the decision of the federal cabinet a year ago that it would support no further large research projects until the 1990s.

Riesenhuber also argues, in further support of his unwillingness to participate in Hermes, that it would be senseless to invest in an "outdated technology", implying that Hermes is a copy of the US shuttle system. He compares the Hermes concept unfavourably with other schemes for the development of reusable space vehicles, such as the British Aerospace HOTOL concept. There is also, in Riesenhuber's mind, anxiety that the French project assumes continued use of the launching site at Kourou, in French Guiana, despite the "explosiveness" of the political climate there.

The dispute between the ministers appears to spring from the decision at the most recent meeting between the federal

Such an emphasis on the priority of the comprehensive programme is not, however, without its risks. President Ceausescu of Romania has already criticized the programme, suggesting that the planners should first have implemented what they undertook to do in June 1984.

What Ceausescu was referring to is not entirely clear, but he may well have meant the much publicized decision in 1984 to raise the economies and technological bases of the "less developed" Comecon countries (Mongolia, Cuba and Vietnam), to the level of the rest; the comprehensive programme says nothing specifically about these countries although the benefits they might expect were mentioned at the press conference afterwards. **Vera Rich**

Chancellor, Helmut Kohl (CDU), and the President of France, François Mitterrand, that the two governments should sponsor a joint study project aimed at turning Hermes into a practical machine.

Riesenhuber's coolness on Hermes conflicts with the technical opinion given to him some months ago by the German Air and Space Research Institute (DFVLR), which recommended participation. But the minister points out that the DFVLR report drew attention to several technical defects of the French proposal such as the inability of the new Ariane V rocket system to compete economically with the new US shuttle-based technology and the intended polar orbit of Hermes, which would be incompatible with the orbits of the US space stations. The French plan would also require new emergency landing sites and data transmission networks. But Riesenhuber may have been chiefly influenced by the consideration that West Germany hopes to influence the development of an independent European space technology would be undermined if the funds were to be paid directly to the French.

The DFVLR proposals for a new West German initiative in advanced space technology are ambitious. The institute recommended spending about DM600 million a year on a three-cornered programme including both Hermes and the proposed European space vehicle "Columbus", intended as an adjunct to the proposed US space station.

Riesenhuber says that his research budget cannot cover costs on this scale, and is urging that any programme of this kind sponsored by the federal government should be partly financed by industrial sources, perhaps to the tune of 20 or 30 per cent. Riesenhuber points out that many of the proposed experiments could be of industrial value to German industry.

Jürgen Neffe

Sakharov's scientific legacy

SIR—Erast B. Gliner is right to say that Sakharov's contribution to science¹ should be emphasized in the campaign to end his exile and to save his life. However, Gliner's statement that Sakharov "is not considered as a head of some scientific school inside of the Soviet Union or abroad" is not entirely correct. Sakharov's pioneering work on the problem of a controlled thermonuclear reaction, which began the well known tokamak project, was the main reason for his election as a full member of the Academy of Sciences of the USSR in 1953, together with his co-author Igor Tamm. More than 20 years later, Sakharov was elected as a member of the US National Academy of Sciences for similar reasons. In a brief autobiography² published in 1974, Sakharov made a special point about two projects that he considered the most important of his contributions to nuclear physics:

In the summer of 1950, almost simultaneously with the beginning of work on the thermonuclear weapon, I. E. Tamm and I began work on the problem of a controlled thermonuclear reaction; i.e., on the utilization of the nuclear energy of light elements for purposes of industrial power. In 1950 we formulated the idea of the magnetic thermo-isolation of high-temperature plasma, and completed estimates on the parameters for thermonuclear synthesis installations. This research, which became known abroad through a paper read by I. V. Kurchatov at Harwell in 1956 and through the materials of the First Geneva Conference on the Peaceful Use of Atomic Energy, was recognized as pioneering. In 1961 I proposed, for the same purposes, the heating of deuterium with a beam from a pulse laser. I mention these things here by way of explaining that my contributions were not limited to military problems.

In the Soviet Union, acknowledgment of Sakharov's work has been made only in publications from before 1968. Even though the tokamak project for which I. Tamm and A. Sakharov laid the foundations is continuing in the Soviet Union (the tenth, eleventh and twelfth tokamaks are now operating for research purposes), Sakharov's name has been omitted from the history of this field. Sakharov's role was last acknowledged in two official biographies of Igor Kurchatov published in 1967^{3,4}. I. Golovin, a close colleague of Kurchatov (the head of the Soviet atomic project), recorded Kurchatov's reaction to Sakharov's theory in a very clear way: "Kurchatov was sitting in his study on New Year's Eve 1950 when he turned [to Golovin] and said: 'Sakharov has boosted us to tackle the second atomic problem of the twentieth century which is no less magnificent [than fission] — obtaining boundless energy by burning the waters of the ocean' ". In some Western histories of Soviet science, the role of Sakharov in the tokamak project is fully acknowledged⁵.

When asked about Sakharov's fate, some Soviet officials (including Anatoly Alexandrov, president of the Academy of Sciences of the USSR) normally answer that Sakharov is restricted to Gorky because he is in the possession of important military secrets and that this exile is made in strict observance of Soviet laws. Neither reason is correct. Sakharov was exiled to Gorky on the basis of an "individual" decree signed by the late President Leonid Brezhnev in 1980 as a reprisal for Sakharov's protest over the Soviet invasion of Afghanistan. There are no provisions in Soviet law for such a form of punishment, and the exile was imposed without any trial or decision of the court. Sakharov had had no access to classified information since 1968.

Now, when the new leadership clearly considers many aspects of Brezhnev's internal policy in a very unfavourable light, his legal actions may also be re-examined more carefully and critically. Gorbachev's intention to accelerate scientific and technological cooperation between East and West could not be served better than by restoration of full freedom to Sakharov.

ZHOES A. MEDVEDEV

National Institute for Medical Research,
Mill Hill, London NW7 1AA, UK

1. Gliner, E.B. *Nature* **318**, 513 (1985).
2. Sakharov, A.D. *Sakharov Speaks*, 30-31 (Knopf, New York, 1974).
3. Astashenkov, P. *Kurchatov*, 173 (Moldaya Gvardija, Moscow, 1967).
4. Golovin I.N. *Kurchatov* (Atomizdat, Moscow, 1967).
5. Parry, A. *The Russian Scientist*, 172-173 (Collier Macmillan, London, 1973).

Velikovsky

SIR—In his letter (*Nature* 10 October, p.470) criticizing the discussion of the Babylonian Venus observations in my review of Bauer's *Beyond Velikovsky*, Lynn E. Rose claims that he and Vaughan have worked to vindicate these data whereas Huber has rejected two-thirds of them.

The Babylonian texts in question were copied a millennium after the observations; there are so many variants between the copies as well as self-contradictions within individual tablets that at least 20 per cent of the data is suspect from the outset. As one of the most enthusiastic Velikovskians, Rose wishes to use these questionable data to prove that the Earth's orbit has changed dramatically in the past three millennia. Huber, as a scientist inclined to believe in the stability of the Earth's orbit, wishes instead to show that this corpus of material is statistically consistent with modern parameters, and even after throwing out the demonstrably bad sections of the text, over half of the 49 observations agree with modern values

within the accuracy of the Babylonian observations.

C. Leroy Ellenberger, no longer a convinced Velikovskian, has pointed out to me that I might nevertheless have even better cited the uniformity of Greenland ice core Dye 3 as a way in which science could actually demonstrate that Velikovsky's scenario did not happen. This 2,000-metre sample is continuous and datable for the past 10,000 years and shows no dust or acid layers that would signal the sort of universal catastrophe predicted by Velikovsky.

OWEN GINGERICH

Harvard-Smithsonian Center for
Astrophysics,
Cambridge, Massachusetts 02183, USA

Metric system

SIR—While endorsing all the comments made by your correspondent Alex Berezin (*Nature* **317**, 762; 1985), I hope that a mixture of different systems of units, including some nonmetric ones, will be retained, at least in the field of aviation. It is a major contribution to safety for a pilot to know that data quoted by radio in feet refer to altitude, while data quoted in metres refer to visibility, and that nautical miles are used to refer to distance along a route, from a radio beacon or airport, etc.

This system is standard throughout the world, the only exception being, characteristically, the United States, where statute miles are thrown in for measuring both visibility and distances, runways are measured in feet instead of metres and air pressure is quoted in inches (of mercury) instead of hectopascals.

If one overlooks this idiosyncrasy, the system works most satisfactorily and is very well suited to be used throughout international airspace, a space which is non-Euclidean, anisotropic and subject to changes of scaling factors (wind, air-pressure and so on) at any time.

ROBERT BYWATER

Department of Cell Research,
Wallenberg Laboratory,
University of Uppsala,
Uppsala, Sweden

Nuclear weapons

SIR—Edward Teller's letter (*Nature* **318**, 99; 1985) is accompanied by two graphs, one of relative numbers and the other of relative yields of US nuclear weapons. In both cases, the data presented end in 1980, before President Reagan's accelerated weapons programme, with which Dr Teller was closely associated and of which he should be in a position to present the consequences, both for numbers and yield. What are the figures for the past two years?

A. J. McEvoy

1015 Ecublens,
Lausanne, Switzerland

Pragmatism is not enough

SIR—It is a well-publicized fact that many species of organisms are in danger of extinction from human activities, especially in the humid tropics where massive deforestation threatens to exterminate countless forms not yet discovered by science. Fortunately, many individuals and organizations have risen to oppose the shortsighted economic and social behaviour that causes this problem — above all, the continued growth of human populations. Many arguments have been adduced for the importance of species preservation, including the actual or potential value of species as food or genetic resources, agents of biological control, components of healthy ecosystems and of our own life-support systems, or objects of aesthetic or intellectual interest. E. O. Wilson, in his recent book *Biophilia*, offers a more subtle argument: the innate affinity of man with other living things, and the fundamental connection between the value we place on them and the value we place on ourselves.

All these arguments are valid, and worth making at every opportunity. However, I suggest that we make a serious and possibly fatal tactical error when we rely solely on such justifications for species preservation. They are all based on the same flawed assumption: that the survival of a species is ultimately justified by its usefulness to man.

Take, for example, the popular argument that the humid tropics are vast untapped reservoirs of potentially valuable genetic diversity. This is true, and among the better reasons for preserving the rainforests. But what will happen to this justification if advances in genetic engineering in the next century make wild gene pools superfluous (or seemingly superfluous) for practical applications? The universal tendency of our technology, after all, is to replace natural products with synthetic ones, and to reduce our dependence on wild species of every sort.

When scientists try to oppose polluters, exploiters and developers solely with these kinds of pragmatic arguments about the value of species, ecosystems or gene pools to human well-being, we have already been manoeuvred into fighting on the ground they have chosen. Our pragmatic arguments for the long-term value of species will be weighed against their pragmatic arguments for the immediate needs of human beings. If an impartial judge rules that their arguments are more compelling and that a flood-control dam will objectively provide more tangible benefits to humanity than will an endangered species, to whom will scientists appeal?

If man's momentary need is allowed to decide the fate of other species, then in the

long run the odds are stacked against them. Yet as scientists we owe our allegiance to objectivity and therefore to pragmatism. This, in my view, is why we cannot afford to speak only as scientists, or to let ourselves be limited by that label. We are scientists second and human beings first, and therefore must judge and act and justify our actions according to criteria that transcend science and pragmatism; namely, moral criteria.

If we are ultimately to preserve our biological inheritance, the only position we can take that is sufficiently strong — the only ground that is sufficiently high to be defended against the pragmatists, the economists, the politicians and the exploiters — is that we humans, as the *de facto* stewards of nature, are morally accountable for our stewardship. At the very least, we are accountable to posterity, and for such blunders as we have already committed with toxic and radioactive wastes, we may even live to be cursed by our own children. Those who acknowledge an authority above and beyond this world have still more reason to fear judgement. But if we pride ourselves on any claim to morality at all, whatever, its philosophical basis, that morality must lead us to use nature in a way that will preserve its diversity permanently intact.

This, admittedly, is a moral principle that is more implicit than explicit in many of the theologies and philosophies of the past. But just as there is progress in scientific understanding, there is also progress in moral philosophy and in what we are pleased to call "civilization". Little by little, the human race has grudgingly come to admit that certain things are simply wrong — such as murder, theft, slavery, torture, racism, genocide and even cruelty to animals. Though still committed, these are generally acknowledged, at least, to be crimes. Extermination of a species is also a crime. It is murder of a unique and irreplaceable evolutionary "individual"; it is theft at the expense of our own posterity; and it is a form of genocide as indefensible as any other.

These are the facts we must force mankind to admit, and ultimately to codify in enforceable international law; and there is no time to waste.

DARYL P. DOMNING

Department of Anatomy,
Howard University,
Washington, DC 20059, USA

Bees in SE Asia

SIR—As people of, we believe, "some importance", we read with dismay your editorial on "Bees in South-East Asia" (*Nature* 317, 190; 1985). Scientific thinking on this issue is clouded by political

rhetoric on both sides. We wish to protest about a different matter — your judgement that "nobody of much importance seems to have taken the biological warfare scare seriously".

We take it seriously, as do some of our patients — the H'Mong people of Laos. It is our privilege to work at a hospital which cares for these surviving refugees. They know, perhaps better than any of us, just how seriously to take it.

Atrocities are indeed hard to imagine. To suggest that these people, who believe that biological warfare has been used against them, are "nobody of much importance", rings of past atrocities which also were denied.

We call on you to be more circumspect in conferring the mantle of importance on some people and not on others. And we caution that science is not well served by the assumption that the "important" people are more likely to be astute observers or logical thinkers.

KAREN R. REEVES

WILLIAM H. ANDERSON

St Elizabeth's Hospital,
736 Cambridge Street,
Boston, Massachusetts 02135, USA

Leprosy vaccine

SIR—Your report (*Nature* 317, 665; 1985) about the proposed test of the World Health Organization (WHO) leprosy vaccine in India does not accurately represent the status of the method used to prepare the vaccine. Although this has not been formally published, the protocol is freely available from WHO and elsewhere and involves no proprietary or patented processes. Further, I have demonstrated the process in two separate laboratories in India, both of which have all the necessary apparatus and chemicals. Several visitors from India have also been shown how to purify *M. leprae* by the process. So it is not clear what technology needs to be transferred. The involvement of the company Wellcome in the matter is simply because materials for use in man must be prepared in licensed premises; there is no question of either development or exploitation of the process by Wellcome.

Your correspondent correctly comments that the main problem of indigenous production of the WHO-type vaccine is that the armadillo colony set up by the Indian Council for Medical Research failed to thrive. These are difficult animals to keep, but there is no restriction on advice and training on how to keep them.

There may indeed be instances where "multinationals" have restricted access to products by developing countries, but the leprosy vaccine is not such an instance.

PHILIP DRAPER

National Institute
for Medical Research,
Mill Hill, London NW7 1AA, UK

Voyager 2 swings by Uranus

The US spacecraft launched towards Jupiter 8.5 years ago is about to encounter Uranus, a planet that has completed only two of its orbits since it was first discovered. The event could be exciting.

THIS time two weeks from now, on 24 January, the US space probe Voyager 2 will make its closest approach to the planet Uranus, providing many people with a reminder of a truth too easily forgotten, that for all its supposed familiarity, the Solar System is huge. The most striking sign of that is that Voyager 2 will have been on its way for more than eight years by the time it passes within 81,500 km of Uranus. Another is that Uranus, unlike both Jupiter and Saturn, is so far away (3×10^9 km) that it remains a bundle of conjectures that are unlikely to be resolved by a single spacecraft.

Nobody should be surprised. That it is just two centuries since Uranus was discovered gives the show away. In an orbit whose radius is 19.18 times greater than that of the Earth, Uranus intercepts only four per cent of the sunlight reaching the Earth even though its radius is 4.02 times as great. At that distance, the image is tiny (1.8 arc seconds across), small enough to fit inside the Great Red Spot on the surface of Jupiter.

The real surprise is what it was in 1781, that the discovery of Uranus should have fallen to German-born William Herschel, the professional oboe-player and musician who (like Handel) had followed the Hanoverians to Britain in search of a living, and who seems to have become an amateur astronomer by accident. The tale of how Herschel's gifted amateurism, which turned on his flair for making accurate mirrors from speculum metal, excited the enthusiasm of Georgian England was nicely told at the International Astronomical Union's symposium at Bath on the bicentenary. The way in which the irregularities in the orbit of Uranus led directly to the discovery of Neptune is another heroic tale. But for the rest, nobody is much wiser about Uranus than in Herschel's time.

Inference rather than direct observation remains the chief source of what little is known; none of the occasional reports of surface features on the planet is regarded seriously. Even the still startling intelligence that the spin axis of the planet is virtually parallel to the plane of its orbit derives most directly from the observation that the orbits of the five known satellites (the first two found by Herschel) lie in planes that are virtually perpendicular to the plane of the planet's orbit, although the measured oblateness (0.022, with an uncertainty of about 5 per cent), which

varies with the phase of the planet at observation, fits in with the supposed spin axis. More recent attempts to learn something of the motion of the planet from Doppler measurements of atmospheric emission lines have helped only to pin down the period of rotation to about 15 hours (and few will be surprised if Voyager 2 recovers a different value).

This is why the next few weeks may bring more information about Uranus than the whole of the past two centuries. The hope is that the set of eleven experiments that gathered a rich harvest of information from the successive encounters with Jupiter (in 1980) and Saturn (in 1981) will still be functioning at Uranus (and at Neptune, which will be encountered on 24 August 1989, almost exactly twelve years after Voyager 2 was launched).

The visual observations may be even more telling on this occasion than at the previous encounters with outer planets. For if there are recognizable features on the surface of Uranus, it should at least be possible to learn directly something about the speed of revolution of the atmosphere of the planet (which is not necessarily that of the bulk). Indeed, the three cameras on Voyager 2 should be capable of yielding a resolution of better than 700 km for 700 hours before and after the encounter, which means that the daily newspapers may have splashed the first photographs of Uranus before this issue of *Nature* has appeared.

Another of the planned routes to a direct measurement of the speed of revolution may be a disappointment. If Uranus has a permanent magnetic field like that of the Earth, there will also be a magnetosphere that dominates the solar wind and, within that, a store of charged particles trapped in the magnetic field whose interaction with the atmosphere will give radio emissions varying with the rotation of the planet (if the magnetic and rotation axes are different). The first reports from what is called the Planetary Radio Astronomy Experiment suggest only meagre radio emission, at best. Perhaps there is no magnetic field worth speaking of. The next few days should tell.

This question has a bearing on the chemical constitution of Uranus and thus on current versions of the theory of how the planets were originally formed. Outwardly, Uranus and Neptune are similar. Uranus has 14.6 Earth masses, Neptune has 16.78. But the radii are oppositely related,

at 4.02 and 3.89 Earth radii respectively. The difference of density may not be great, but it requires an explanation.

The standard assumption is that, even at these great distances from the Sun, planets will contain substantial quantities of the elements whose refractory oxides finish up as rocks on planets like the Earth. But Uranus and Neptune will have two other kinds of materials, those that form ices at the temperatures that reign (the atmosphere of Uranus, colder than that of Neptune, appears to be at a mere 65 K) but also materials that are still gaseous under those conditions, hydrogen and helium most significantly.

Given such a model, together with the mass and radius of the planet, it is possible to work out the relationship of pressure and density with depth beneath the surface. If the planet is spinning on an axis, it is also possible to relate the equatorial bulge and the aspherical components of the gravitational field to the speed of rotation, whence several attempts to calculate the rotational speed. (The shape of the gravitational field is inferred from, for example, the motion of the satellites or the shapes of the rings, of which, at Uranus, there are nine.) But here again, the data are not good enough to settle unambiguously the several persisting uncertainties. Here again, Voyager 2 should help enormously, not simply because its trajectory near Uranus is well designed for helping to pin down the gravitational field but because there will be direct measurements of the composition of the atmosphere.

That raises several questions. The ratio of deuterium to hydrogen should be greater than on the Sun, but its measured value may be a guide to the temperature at which these planets formed. Why there is virtually no ammonia in the atmosphere of Uranus is another puzzle. (Methane is abundant.) The narrowness of the nine rings, and their apparent opacity, is another problem (but see this issue, p. 115) on which some light may be thrown by the very close approach to the innermost satellite Miranda expected for Voyager 2, a mere 15,000 kilometres. Whether there is an internal source of heat other than that of energy released by minor gravitational readjustments within an apparently stable planet may also be determined. The way things have turned out, the spacecraft is an instrument of which even Herschel would have been proud.

John Maddox

Ocean ecology

Light and ultraphytoplankton

from G. E. Fogg

H. LOHMANN, studying filter mechanisms used by planktonic appendicularians to collect extremely fine particulate matter from sea water, pointed out the inadequacy of the conventional fine silk net for sampling plankton. He suggested that the small organisms which pass through the net could be important in the economy of the oceans because they might multiply much faster than the larger forms. The smallest of the phytoplankton, bacteria and tiny protozoa, are now grouped as picoplankton (diameter 0.2–2.0 μm) or ultraplankton (diameter less than 10 μm). The behaviour of these microorganisms is analysed in this issue (Glover, H.E., Keller, M.D. & Guillard, R.R.L. *Nature* **319**, 142; 1986) and was the subject of a recent NATO advanced study workshop. The conclusions support Lohmann's 75-year-old suggestion and mean that our ideas about ocean ecosystems will have to be revised.

Although it has often been noted that much of the photosynthetic activity of water samples is contained in the fraction that passes through the phytoplankton net, these minute organisms have remained largely unconsidered because they are so difficult to identify and count. Picoplankton only leaped into prominence when the value of the epifluorescence microscope for counting tiny coccoid cyanobacteria (blue-green algae) was realized (Johnson, P.W. & Sieburth, J. McN. *Limnol. Oceanogr.* **24**, 928; 1979 and Waterbury, J.B., Watson, S.W., Guillard, R.L. & Brand, L.E. *Nature* **277**, 293; 1979). Because they contain biliprotein pigments, these organisms are easily recognized by their strong and characteristic autofluorescence. Epifluorescence shows that they are abundant in most, if not all, the world's oceans, and the filter fraction in which they are collected contains most of the total photosynthetic capacity.

Physical considerations suggest that organisms of this size are peculiarly suited to planktonic life. Their sinking rates are negligible compared with the scale of the turbulence found in even the calmest seas. They have high surface/volume ratios which, combined with the steep diffusion gradients that are set up around very small sinks, allow them to take up nutrients at a high rate. Furthermore, a given amount of photosynthetic pigment dispersed in small cells absorbs light more effectively than an equivalent amount packaged in large cells. The strains examined in laboratory culture grow best at low light intensities of less than one fiftieth of full sunlight. Perhaps the only disadvantages of small

size for a phytoplankter are the difficulties of moving into waters richer in nutrients or more favourably illuminated, and a greater vulnerability to predators.

Picoplanktonic cyanobacteria are generally found in concentrations of 10^4 cells per ml in inshore and offshore waters but are more evident in nutrient-poor offshore waters where larger phytoplankton, less able to use low concentrations of nutrients, are not successful. Although they are found throughout the water column in the euphotic zone, these cyanobacteria are concentrated at the bottom of the zone. This is not because they sediment out but is rather because they grow best in these conditions, using the low irradiance efficiently to support growth and benefiting from nutrients transported up from richer waters below. The oceanic picoplanktonic cyanobacteria contain large amounts of phycoerythrin, a pigment which seems admirably suited to absorb the blue-green light penetrating into deep water, but most of this pigment seems to be photosynthetically inactive, as demonstrated by its high fluorescence yield. Phycoerythrin may be more important as a nitrogen reserve than as an accessory photosynthetic pigment (Wyman, M., Gregory, R.P.F. & Carr, N.G. *Science* **230**, 818; 1985).

As well as cyanobacteria, there are eukaryotic ultraphytoplankton, detectable by the autofluorescence of their chlorophylls. These have so far scarcely been

characterized and are difficult to grow in culture, but they are sometimes more abundant than the cyanobacteria in deep water and seem to grow best at even lower irradiances. Glover and her colleagues have now examined the photosynthetic performances and growth efficiencies of such forms at different irradiance levels and in different wavebands and find that the eukaryotes do better than the cyanobacteria in the dim blue-violet light that penetrates to the bottom of the euphotic zone in clear oceanic waters. Chlorophylls *b* or *c*, the major accessory pigments of these organisms, promote absorption in this region of the spectrum. In contrast, cyanobacteria grow best in green light. The larger eukaryotic phytoplankton have lower efficiencies at low irradiances than the ultraplanktonic species, probably as a result of the size effect discussed above.

It appears that eukaryotic ultraplankton, being adapted to low light intensities, can occupy a zone in which an ample nutrient supply is maintained by transport upwards from deeper water whereas the cyanobacteria need higher irradiances but can persist in waters which are sometimes depleted of combined nitrogen, and perhaps phosphorus, because of their high storage capacity. Further investigation of the physiology of these highly successful tiny organisms, together with increasing knowledge of the activities of the heterotrophic ultraplankton, will help to complete a picture of one component of an ecosystem that is probably responsible for most of the energy transformations and recycling of materials taking place in the oceans. □

G.E. Fogg is at the Marine Science Laboratories, University College of North Wales, Menai Bridge, Anglesey LL59 5EH, UK.

Cell biology

Life without clathrin

from James E. Rothman

DURING receptor-mediated endocytosis, the process by which many macromolecules are taken up from the extracellular fluid into cells, receptors cluster in specialized regions of the cell surface, which invaginate to form coated vesicles. The coating is made of protein and the best characterized of the proteins is clathrin. It has always been assumed that the spontaneous assembly of clathrin molecules into a polygonal array on the surface of the budding vesicle is responsible for driving the budding process. This has also been seen as attractive paradigm for the mechanism of vesicular transport between intracellular compartments. But does clathrin play that role in intracellular transport? Do clathrin-coated vesicles mediate biosynthetic intercompartmental

protein transport, such as the distribution of newly synthesized proteins from the endoplasmic reticulum via the Golgi stacks to plasma membranes, secretory granules and lysosomes? Three recent papers offer some new and surprising answers to these fundamental questions¹⁻³.

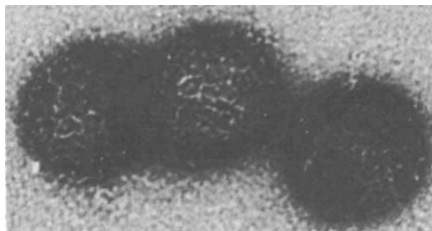
From immunofluorescence studies using antibodies to clathrin it has been clear that clathrin is localized both to the Golgi complex and to the plasma membrane, suggesting a role for clathrin in both biosynthetic and endocytic protein traffic. But an important extension of such studies by Griffiths *et al.*¹ and Orci *et al.*² has now greatly limited the steps in biosynthetic protein traffic that might be mediated by clathrin-coated vesicles and has led to the discovery of a new type of

morphologically coated vesicle, which seems not to contain clathrin. Using colloidal gold-based immunocytochemistry combined with electron microscopy to localize clathrin antigens in the Golgi complex, both groups find that clathrin is mostly concentrated at the trans-most cisterna of the Golgi stack.

The Golgi stack is an asymmetrical structure comprizing a series of distinct compartments or cisternae. Proteins arrive from the endoplasmic reticulum at one end (the 'cis' face) of this stack, pass from one compartment to the next, and finally emerge from the last cisterna at the opposite ('trans') end. Thus, the restriction of clathrin-coated vesicles to the trans-most cisterna suggests that, at most, clathrin-coated membranes serve as carriers from this location to terminal destinations, such as the cell surface, secretory granules and lysosomes.

It is generally agreed that clathrin probably coats primary lysosomes and newly formed secretory granules in the cell. By contrast, a protein that is known to be destined for the plasma membrane (the vesicular stomatitis virus-encoded G protein) could not be detected by Griffiths *et al.*¹ in the clathrin-coated buds forming from the trans-cisterna. The picture that seems to be emerging is that clathrin-coated vesicles are restricted to routes connecting the Golgi with some terminal destinations of biosynthetic traffic, but perhaps not the plasma membrane. Lysosomal enzymes and secretory proteins destined for storage granules may be selectively removed from the Golgi in clathrin-coated membranes. Perhaps the left-overs — consisting of cell-surface proteins and constitutive (unregulated) secretions — are transported to the plasma membrane by other types of vesicles, whose content may not be nearly so selective. Of course, the picture may be still more complicated, with polarized cells having multiple surface domains.

Given that clathrin is restricted to the Golgi exit point, how do transported proteins move from the endoplasmic reticulum to the Golgi, and from one cisterna to another across the stack? Another surprise described by Griffiths *et al.*¹ is that although there are morphologically coated vesicles or buds at all levels of the Golgi, they are not labelled by antibodies to clathrin. As the authors point out, the simplest explanation is that these coated structures are not made up of clathrin. The clear and exciting implication is that a new type of coated vesicle, whose coat protein is yet to be discovered, carries biosynthetic protein traffic up to the trans-most Golgi cisterna. A number of mysteries are created. Why is the same type of clathrin coat not used at all stages of biosynthetic protein transport? Are the new type of coated vesicles capable of selective protein transport, like clathrin-



Clathrin-coated vesicles from chicken oocytes. $\times 125,000$, 1% uranyl acetate stain. Photograph courtesy of B. Pearse.

coated vesicles, or might they have quite different functions, perhaps even as bulk carriers? In this connection, note that the first station where a choice of destination must be made along the biosynthetic protein-transport pathway is probably in the trans-most cisterna of the Golgi stack, which is where clathrin is first found.

Yet another surprise is that some cells can manage quite well without any clathrin at all. This sobering finding emerged when Payne and Schekman³ deleted the single clathrin gene from yeast. Yeast has coated vesicles and its clathrin, like mammalian clathrin⁴, is composed of three-legged 'triskelions' containing equimolar amounts of a heavy chain (190 kD in yeast) and a light chain (36 kD)³. Payne and Schekman prepared an antibody against the yeast heavy chain and used this to isolate the heavy-chain gene from a library of yeast DNA in a phage lambda expression vector. Using what are now standard methods in yeast genetics, they replaced the normal clathrin heavy-chain gene of yeast with a grossly deleted version, prepared *in vitro* using restriction enzymes. Spores carrying only the deleted clathrin gene do not synthesize any clathrin heavy chain or even any immunologically cross-reactive material, yet they are quite unexpectedly viable, although they grow more slowly than wild-type cells. The only indication of a defect in the cells is an accumulation of vesicular structures in the cytoplasm and the presence of

a larger than usual intracellular pool of invertase, which, however, is secreted at essentially normal rates.

What could this mean? As the authors suggest, one possibility is that although biosynthetic transport does not require clathrin-coated vesicles in yeast, endocytosis still does. If so, this would fit well with the evidence from animal cells that clathrin may not be needed for constitutive transport from the endoplasmic reticulum through the Golgi complex to the cell surface. (It is not known whether proper vacuole formation is needed for yeast to grow; also, yeast does not store secretory products.) Another possibility would be that there is a 'back-up' system — an alternative clathrin-like molecule that can replace clathrin when needed, or an alternative pathway that can salvage transport. If so, the back-up coat protein must be structurally dissimilar from clathrin proper, since there is but a single clathrin gene and no cross-hybridizing DNA even under conditions of low stringency, nor any other polypeptide that cross-reacts with antibodies to clathrin. A final, and even less comfortable, possibility is that clathrin is not required to form the vesicles that it normally coats, nor for their utilization. This would be truly heretical but cannot be dismissed out of hand.

These unexpected developments add a breath of fresh air and excitement, and suggest that we are about to obtain a more sophisticated view of the process of protein sorting. They also serve as a reminder that generalizations in cell biology can be more dangerous to the health of the investigator than to that of the organism. □

1. Griffiths, G., Pfeiffer, S., Simons, K. & Matlin, K. J. *Cell Biol.* **101**, 949 (1985).
2. Orci, L., Ravazzola, M., Amherdt, M., Louvard, D. & Perrelet, A. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5385 (1985).
3. Payne, G. & Schekman, R. *Science* **230**, 1005 (1985).
4. Mueller, S. & Branton, D. J. *J. Cell Biol.* **98**, 341 (1984).

James E. Rothman is in the Department of Biochemistry, Stanford University, California 94305, USA.

Geology

Are komatiitic lavas voracious?

from Robert W. Nesbitt

KOMATIITE lavas are highly magnesian silicate rocks which are mainly restricted to the first half of the Earth's history. First recognized less than twenty years ago, in the 3,500-Myr-old Barberton greenstone belt in South Africa¹, they have since been identified in most continents where volcanic greenstones are exposed. They are particularly interesting for their restricted but economically important association with Fe-Ni-Cu sulphides: the most important deposits always occur at the base of the first flow of any komatiite lava pile². Huppert *et al.*³ have put forward a model

in which this association is explained by thermal erosion. In a paper on page 136 of this issue, D.I. Groves and colleagues provide an example of this but one which, paradoxically, reinforces previous criticisms of the model⁴.

Komatiite flows consist of an underlying olivine cumulate zone overlain by a zone of skeletal (or spinifex-texture⁵) olivine — that is, olivine set in a matrix of skeletal clinopyroxene and glass. Overall, the grain size and morphology of the olivine changes from a random arrangement of olivine plates with fine-grain size at the

top of the flow to a coarse-grained parallel plate-like arrangement just above the cumulate zone (Fig. 1a, b). These textures, together with geochemical and experimental data, have led to the concept that komatiitic liquids erupted at temperatures of 1,600 °C, some 300–400 °C above those observed for basaltic flows⁷.

Based on these data, Huppert *et al.*³ speculated that, because of their low viscosity, komatiite liquids would display turbulent flow rather than the laminar flow typical of lower-temperature silicate melts. Their calculations suggested that this would result in high temperatures being maintained at the base of flows, so that the komatiite liquids would be capable of thermally eroding the underlying rocks. Consequently, the komatiite flows might create their own channels and, as a result, assimilation of underlying material could take place.

This new concept also provided an explanation for the occurrence of Ni–Fe–Cu sulphides at the base of komatiite flows. If sulphide-bearing sediments were assimilated they would immediately form an immiscible sulphide liquid, because sulphur has low solubility in silicate melts. The liquid would then scavenge Ni and Cu out of the komatiite melt and the resultant Ni–Fe–Cu sulphide liquid would settle into topographical lows at the base of the komatiite flow, which itself was still molten. Although the sulphide–silicate immiscibility model was not new, this scheme provided an explanation for the origin of the sulphur and for the channel or trough-like structures, which are a prominent feature of almost all komatiitic nickel sulphide deposits.

In a further twist to the complexities of komatiite-sulphide genesis, Groves *et al.*⁴ now suggest that the thermal-erosion model only works if a pool of sulphide liquid already exists — the sulphide providing the high thermal-conductivity medium necessary to initiate melting in the underlying rock. Their evidence comes from the hanging-wall ore bodies at Kambalda, Australia, which are unusual in that they are found between flows higher in the section rather than at the base of the first flow. Groves and colleagues have examined spinifex-textured olivine–sulphide associations that occur in komatiite

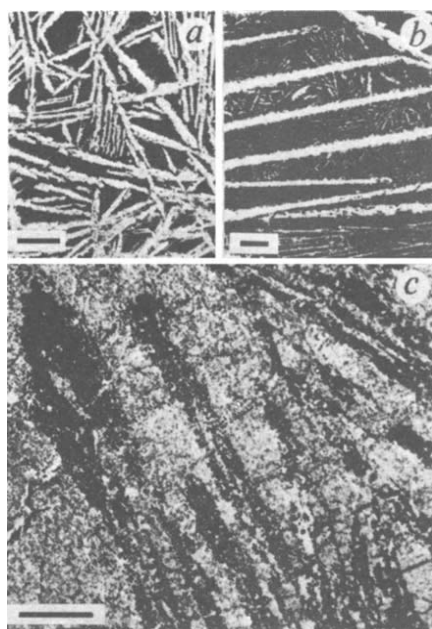


Fig. 1 a, Thin section of a komatiite with random spinifex texture. Olivine (white) plates separate areas of glass and clinopyroxene (dark areas). This textural type is commonly found close to the upper surface of flows. b, Thin section of a komatiite with plate spinifex texture. The long light-coloured crystals are olivine with the inter-olivine areas occupied by clinopyroxene and glass. This is found near the upper surface of komatiite flows but below the random spinifex type shown in a. c, Polished rock slab showing skeletal olivine crystals (dark) and Fe–Ni–Cu sulphide. These may represent pre-existing plate spinifex-textured komatiite⁴.

flow-top zones, just below the massive hanging-wall ore bodies. In these occurrences (Fig. 1c), sulphide occupies the inter-olivine areas; the authors claim that this represents a replacement of the pyroxene and glass that normally occurs there. The main evidence seems to be the occurrence of types transitional between all-sulphide and all-silicate inter-olivine material, interpreted as the result of selective melting in the spinifex zone of the komatiite flow by the transference of heat through the sulphide pool from the overlying komatiite flow. The resulting silicate liquid was then displaced upwards from between the olivine grains by the overlying heavier sulphide, which settled into the space that was previously occupied by pyroxene and glass.

Furthermore, the fact that the olivine texture in the sulphide is always of the plate type, indicates that the fine-grained random spinifex (commonly found above the plate-type) must have been thermally eroded by the overlying flow. This is a particularly critical observation because Groves *et al.* claim that along strike from the sulphide occurrence, the younger komatiite flow directly overlies either a sediment or a spinifex-textured komatiite with random olivine. In other words, thermal erosion occurred only if a pool of sulphide liquid was present. Hence

the paradox — although they provide an example of thermal erosion, Groves *et al.* at the same time caution against the general application of the model.

If komatiite flow on older komatiite does not produce melting without the intervention of sulphide liquid, what about komatiite liquid flowing on to basalt or sediment? Groves *et al.*⁴ have looked again at the major nickel ore environment at Kambalda, where massive sulphides occur in trough-structures separating pillow basalts (footwall) from komatiites. Here the evidence for thermal erosion is less definite, despite the undoubted presence of a major pool of sulphide liquid. The problem lies in the lack of specific marker horizons in the underlying pillow basalt sequence that would indicate whether there has been removal of the basalt. Although they admit that the trough structures almost certainly focused thermal erosion through the presence of the sulphides, the authors insist that such troughs are first-order volcanic/topographical features and not the products of thermal erosion.

We are thus faced with a major dilemma. According to Huppert *et al.*³, sulphide pools are generated by thermal erosion in surface channels through assimilation of sedimentary sulphur, whereas Groves *et al.*⁴ claim that such erosion takes place only under pre-existing sulphide pools. A possible resolution of the problem may be that thermal erosion took place within the crust — evidence from other lines of investigation, particularly studies of zircon ages in the Kambalda area⁸, indicate the influence of crustal contamination. So sulphide contamination of komatiites may have occurred *en route* rather than at the surface.

This solution, however, brings us full circle back to the problem of how to transport a dense sulphide liquid to the surface in a lower-density silicate medium — the outstanding problem in previous models involving mantle-derived sulphide liquids. For academic earth scientists, the tangle of contradictions provides a fascinating research challenge, while exploration geologists continue to puzzle over the problem of why Kambalda komatiites are so richly endowed in nickel sulphides when elsewhere there are only komatiites and sulphidic sediments. □

1. Viljoen, M.J. & Viljoen, R.P. *Spec. Publ. geol. Soc. S. Afr.* 2, 1221 (1969).
2. Gresham, J.J. & Loftus-Hill, G.D. *Econ. Geol.* 76, 1373 (1981).
3. Huppert, H.E., Sparks, R.S.J., Turner, J.S. & Arndt, N.T. *Nature* 309, 19 (1984).
4. Groves, D.I., Korkiakoski, E.A., McNaughton, N.J., Leshar, C.M. & Cowden, A. *Nature* 319, 316 (1986).
5. Clauze-Long, J.C. & Nesbitt, R.W. *Nature* 313, 247 (1985).
6. Nesbitt, R.W. *Geol. Soc. Aust. Spec. Publ.* 3, 331 (1971).
7. Green, D.H. *Geology* 3, 15 (1975).
8. Compston, W., Williams, I.S., Campbell, I.H. & Gresham, J.J. *Earth planet. Sci. Lett.* (in the press).

Robert W. Nesbitt is Professor of Geology at the University of Southampton, Highfield, Southampton SO9 5NH, UK.

100 years ago

In the twentieth volume of the *American Journal of Science* I gave a preliminary account of my search for the trans-Neptunian planet. I regard the evidence of its existence as well-founded, and nothing has weakened my conviction as to its existence in about that part of the sky assigned; while the independent researches by Prof. Forbes conducted him to an identical result. That five years have elapsed, and the planet is still unfound, is not sufficient assurance to me that its existence is merely fanciful. From *Nature* 33 258, 14 January 1886.

Cosmic rays

A supernova for a neighbour?

from Arnold Wolfendale

COULD a supernova have exploded recently on our doorsteps? Don Clayton, D.P. Cox and F.C. Curtis think so. In *The Galaxy and the Solar System* (University of Arizona Press, in the press), they suggest that a supernova explosion occurred 15 pc away — only ten times the distance to the nearest star — about 10^5 years ago. If this controversial claim is correct, it could help to explain the origin of cosmic rays.

It is remarkable that 75 years after they were discovered by Victor Hess, researchers still argue about where cosmic rays come from. The seemingly incessant rain of cosmic rays that bombard the Earth consists of atomic nuclei, protons, electrons and about one part per million of γ rays. This tiny γ -ray component is proving of considerable value to researchers because γ rays travel in straight lines, unlike the particles, which follow tortuous paths in the tangled magnetic fields of the Galaxy. Because many of the γ rays seem to be produced by the cosmic-ray particles interacting with the gas in the interstellar medium (ISM), they can be used to probe the spatial distribution of the particles and thus give a hint as to their sources.

Although some particles achieve enormous energies — up to 10^{20} eV, compared with about 10^{12} eV in the giant accelerators — it is the lower-energy particles that are of concern here. Among the many contenders for their generation, supernovae (SN) — exploding stars, typified by the well-documented Crab Nebula of 1054 — are currently the favourite. Although they were first suggested as cosmic-ray sources in 1934, problems arose because the cosmic ray particles were supposed to lose energy in the ensuing supernova remnant (SNR). Recent advances have, however, included clever ideas on how shock waves can transform shock energy into cosmic rays with high efficiency so that the cosmic-ray energy in a SNR increases rather than falls with time. Support has come from claims that a comparatively nearby old remnant, termed the Loop I SNR, has within it a higher cosmic-ray intensity than elsewhere (Bhat, C.L. *et al.* *Nature* **314**, 515; 1985).

The ideas of Clayton and colleagues on the thorny question of the environment and history of the Solar System are the most recent developments bearing on the origin of cosmic rays. Gone are the days when the ISM was considered to have a uniform density of 1 atom cm^{-3} ; now it is thought to have at least three phases differing in density and temperature. Clayton *et al.* propose that the Solar Sys-

tem is immersed in a 'cloudlet', with a density of 0.1 atom cm^{-3} and temperature 10^4 K extending about 1 pc from the Sun, residing in a medium of much lower density and considerably higher temperature ($4 \times 10^{-3} \text{ atom cm}^{-3}$, 10^6 K), that extends approximately 100 pc from the Sun. It is in this lower-density medium that a SN is assumed to have occurred only 15 pc away about 10^5 years ago.

The authors point out two factors favouring their proposals: the argument of D.P. Cox and P.R. Anderson (*Astrophys. J.* **253**, 268; 1982) that this could explain the observed soft X-ray flux, and Clayton's own suggestion that the comparatively strong γ -ray line from the unstable ^{26}Al nucleus in the ISM comes from the debris of the SN explosion. This γ -ray line is very interesting as it is the first clear case of a line from radioactive nuclei in the general ISM; it was detected by the HEAO-3 and Solar Maximum Mission spacecraft, both of which recorded fluxes in excess of expectation. ^{26}Al has a half life of about 10^5 years and some is doubtless produced in SN explosions; if a SN were so close and had exploded so recently, little ^{26}Al would have been lost by decay, so that the γ -ray flux could be entirely explained.

What effect would such a SN have on the ambient cosmic-ray flux? The authors expect the present value to be twice the average but I find this difficult to accept. There is now a wealth of data on the gradients of cosmic-ray intensity in the Galaxy, obtained by satellite studies of γ rays above 100 MeV, and an excess much more than 30 per cent would be difficult to accommodate, at least in the important proton component. Problems also arise with the relative numbers of cosmic-ray 'nuclei' more massive than protons, in particular with the 'grammage' distribution, which is an indicator of the amount of ISM through which the cosmic rays have passed. Clayton's group are able to get round these difficulties by involving four components of the cosmic radiation that have achieved their energies in different ways in the remnant and in the supernova itself — but this seems a rather contrived solution.

Where, then, does all this leave the problem of cosmic-ray origin? It seems increasingly possible that supernova shocks accelerate a significant fraction of the low-energy cosmic-rays. Although a supernova as recent as 10^5 years ago and as close as 15 pc seems unlikely, some of the ideas put forward by Clayton *et al.* for explaining outstanding anomalies in related areas are worthy of much more detailed study. □

Arnold Wolfendale is Professor of Physics in the University of Durham, South Road, Durham DH1 3LE, UK.

Gene regulation

Importance of helical periodicity

from Tariq Enver and Roger Patient

ATTEMPTS to understand transcription of eukaryotic genes by the enzyme RNA polymerase II have so far focused either on the identification of the sequences that together constitute the gene promoter or on the proteins (*trans*-acting factors) that recognize these sequences and are necessary for transcription. A paper by Takahashi *et al.* on page 121 of this issue¹ demonstrates that the static arrangement of the promoter sequences is an important parameter for transcription. The authors suggest this may best be interpreted as reflecting a role for protein – protein contacts in promoter activation.

Broadly speaking, RNA polymerase II promoters are organized according to a common modular plan². The 'TATA box' specifies accurate initiation of transcription from a position 25–30 base pairs (bp) downstream (of itself). Additional 'upstream elements' important for efficient transcription are located within 100 bp of the transcription start. And transcriptional activity may be further modulated

by an enhancer element. As shown in the figure, the 'early' promoter of simian virus 40 (SV40) conforms to this general scheme. The upstream elements appear in the form of six GC-rich sequence motifs, two each within the three 21 / 22 bp repeats; a pair of 72 bp repeats constitute the enhancer element.

Takahashi *et al.*¹ have tested the effects of altering the stereospecific relationships of these three promoter elements of SV40 by making insertions of odd or even multiples of 5 bp — about half a turn of the DNA helix — either between the enhancer and 21 bp repeats (site P in the figure) or between the 21 bp repeats and the TATA box (site Q). Strikingly, insertions at either site that maintain the helical pitch relationships of the promoter have little effect on transcriptional efficiency, whereas those that alter it reduce transcriptional efficiency by up to 10-fold. One interpretation of the importance of the helical disposition of the promoter elements is that it facilitates contacts between

the *trans*-acting factors that bind at the SV40 promoter elements^{2,3}, and/or that it provides the appropriate recognition surface for RNA polymerase II.

Precedent for the involvement of protein-protein contacts in the regulation of gene expression comes from studies on *Escherichia coli*. The elegant work of Ptashne and coworkers⁴ has clearly demonstrated that whereas repression by bacteriophage lambda repressor (*cl*)

the helix could block access of transcription factors to that face and the extent of blockage would vary with periodicity.

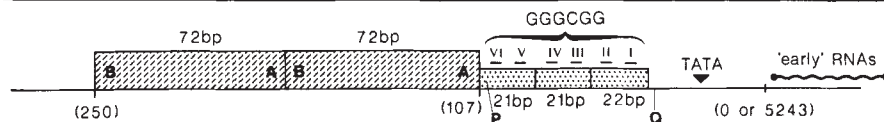
Particularly intriguing is the requirement for stereospecific alignment between the enhancer of SV40 and its 21 bp repeats. Might this shed light on enhancer mechanisms, and can it be reconciled with the fact that enhancers potentiate transcription from their promoters in a way that is essentially independent of orientation,

distance upstream of the promoter, the model is sterically unattractive for a downstream enhancer. An attractive modification to the model is that the loop forms only transiently to facilitate intramolecular factor transfer. But, although upstream enhancement has been demonstrated *in vitro*, downstream enhancement has not, suggesting that simple factor transfer cannot be the whole story. The magnitude of enhancement *in vitro* is only about 10-fold¹⁴ whereas it is 100–1,000-fold *in vivo*. Interestingly, the transcription level seen *in vivo* by Takahashi *et al.* varies with insert size over the range of variation seen *in vitro*. Thus, it is possible that both may be reflecting only one component of the proposed biphasic nature of the 72 bp repeats, whereby they act as both an enhancer and an upstream promoter element at short range but just as an enhancer at long range¹⁵. The helical periodicity and *in vitro* effects may be due to their action solely as a promoter element. It is tempting to suggest that domain A in the 72 bp repeats is the promoter element and domain B is the enhancer. One could then predict that domain B could be completely separated from domain A with little loss of activity; in fact, 50 bp seems to be the upper limit¹². □

1. Takahashi, K. *et al.* *Nature* **319**, 121 (1986).
2. Dynan, W.S. & Tjian, R. *Nature* **316**, 774 (1985).
3. Wildeman, A.G. *et al.* cited in ref. 1 as ref. 16.
4. Ptashne, M. *Trends biochem. Sci.* **9**, 142 (1984).
5. Adhya, S. & Garg, S. *Cell* **29**, 287 (1982).
6. Dunn, T.M. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 5017 (1984).
7. Wu, H.-M. & Crothers, D.M. *Nature* **308**, 509 (1984).
8. Drew, H.R. & Travers, A.A. *J. molec. Biol.* **186**, 1 (1985).
9. Nordheim, A. & Rich, A. *Nature* **303**, 674 (1983).
10. Jackson, D.A. & Cook, P.R. *EMBO J.* **4**, 919 (1985).
11. Serfling, E. *et al.* *Trends genet. Sci.* **1**, 224 (1985).
12. Zenke, M. *et al.* *EMBO J.* (in the press).
13. Irani, M.H. *et al.* *Cell* **32**, 783 (1983).
14. Sergeant, A. *et al.* *J. molec. Biol.* **180**, 577 (1984).
15. Waslyk, B. *et al.* *Nucleic Acids Res.* **12**, 5589 (1984).

Tariq Enver and Roger Patient are in the Department of Biophysics, Kings College London (KQC), 26–29 Drury Lane, London WC2B 5RL, UK.

Schematic diagram of the simian virus 40 'early' promoter.



protein is mediated by steric exclusion of RNA polymerase, the simultaneous stimulation of the adjacent, divergently transcribing promoter for the *cl* gene itself is mediated by protein-protein contacts between the repressor and RNA polymerase. The proximity of the binding sites for activator proteins and RNA polymerase in the galactose⁵ and arabinose⁶ operons also suggests that activation occurs by protein-protein contacts. However, one of these activator proteins, the cyclic AMP receptor protein, acts on several operons, and the portions of its binding sites relative to the promoters are variable, thus calling into question whether induction always works by protein-protein contacts.

What other explanations are possible for the helical periodicity observed by Takahashi *et al.*? One possibility currently attracting attention is DNA bending. From studies of trypanosome kinetoplast DNA, Wu and Crothers have pointed out that the sequence elements causing the axis of the helix to deviate must be arranged at intervals approaching 10.5 bp if the sum of the helical deviations is to result in a bend⁷. Bending may be an important component in defining the binding site of a *trans*-acting factor. Work on the positioning of nucleosomes on DNA⁸ provides evidence that bending is important for protein-DNA interactions and it may well also act to facilitate protein-protein contacts. Such a mechanism has been suggested for the induction of the lactose operon of *E. coli* by cyclic AMP receptor protein, which induces DNA bending on binding to its recognition site and may thereby facilitate contacts with RNA polymerase⁷.

Alternatively, the observed helical periodicity may reflect a requirement in factor binding for the stereospecific alignment of two or more altered DNA structures. Possibly relevant in this context is the identification of Z-DNA segments in the SV40 72 bp repeats⁹. Another quite attractive explanation would involve the interaction of the promoter with the nucleoskeleton¹⁰. Attachment along one face of

distance or position relative to the gene¹¹? In their paper, Takahashi *et al.* cite as yet unpublished evidence from their group¹² that each 72 bp enhancer motif contains two distinct binding domains (A and B in the figure). If, as their data imply, the important protein-protein interaction is between factors bound at A and the 21 bp repeats, then reversing the orientation of the enhancer with respect to the 21 bp repeats might be expected to perturb the critical factor interaction.

Takahashi *et al.* suggest that action at a distance could still be mediated by protein-protein contacts if the interposing DNA is looped out^{2,11}. Possible examples of this type of mechanism have been found in the galactose and arabinose operons of *E. coli*, where it has been suggested that repressor monomers bind at two well-separated sites and monomer-monomer interactions facilitate DNA loop formation^{13,6}. Interestingly, insertions between the two binding sites in the arabinose operon show the same helical periodicity as described by Takahashi *et al.* for SV40.

Although DNA loop-out seems a reasonable model for enhancer action from a

Plate tectonics

Microplates in Antarctica

from A.K. Martin

Two basic schools of thought have emerged on how Antarctica, and particularly West Antarctica, fitted against southern South America before the break-up of Gondwanaland. One suggests that Patagonia and the Falkland Plateau fit into the Weddell Sea, implying that East and West Antarctica have formed one plate since the Jurassic^{1,2}. The other places East Antarctica against Mozambique and overlaps West Antarctica with the Falkland Plateau^{3,4}. This overlap, which was considered unacceptable, implies that West Antarctica has moved relative to East Antarctica and thus formed a separate

plate or several microplates during early break-up⁵. Palaeomagnetic evidence supported the existence of West Antarctica microplates⁶; in this issue, Mitchell *et al.*⁷ provide more evidence for microplates in this region, not from West Antarctica but from the Falkland Islands on the present-day South American plate.

A wealth of seafloor spreading and fracture zone data substantiate that the Falkland Plateau has been part of the South American plate since the initiation of the South Atlantic^{8,9}—its palaeoposition 125 Myr ago is well constrained. The new palaeomagnetic work, however, suggests

that more than 190 Myr ago the Falkland Islands were adjacent to the Transkei coast of South Africa and that a rotation of 180° occurred between 190 and 125 Myr. As originally suggested by Adie¹⁰, this palaeoposition aligns the Lafonian diamictite of the Falklands with the Dwyka tillite of South Africa, which forms the effective demarcation of the northern front of the Cape Fold Belt.

Although the authors concede that much more palaeomagnetic, geochronological and structural work is required to confirm the reconstruction⁷, their work has much wider implications for the geology of the area. First, if the Mozambique palaeoposition for East Antarctica is adopted, then a curvilinear mountain belt (the Gondwanide orogen of Du Toit³) would have stretched from the Sierre de la Ventana in South America across the Cape Fold Belt in South Africa to the Trans-Antarctic mountains^{3,11} (see figure). In their rotated positions, the Falkland Islands and the Ellsworth Block complete the chain — if left unrotated, they would necessitate large re-entrants in the mountain belt. Proponents of a more southerly palaeoposition of a single-plate Antarctica used such re-entrants as criticisms both of this refit and of the philosophy of aligning mountain belts¹². Their objections now fall away.

Debate has also centred around the plate-tectonic nature of the Gondwanide orogeny. The Cape Fold Belt and its intercontinental correlates are seen as constituting a back-arc basin, with the fore-arc and magmatic terranes extending from Chile through Tierra del Fuego to the West Antarctic peninsula¹¹. These latter areas are considered the source of volcanic detritus found in foreland basins on the Gondwanide continents. An alternative view is that the Cape Fold Belt, in particular, represents an intracratonic orogeny; there is a lack of nearby fore-arc or magmatic features, the metamorphic grade is low, and the small amount of crustal shortening is considered incompatible with fast-moving colliding plates^{13,14}.

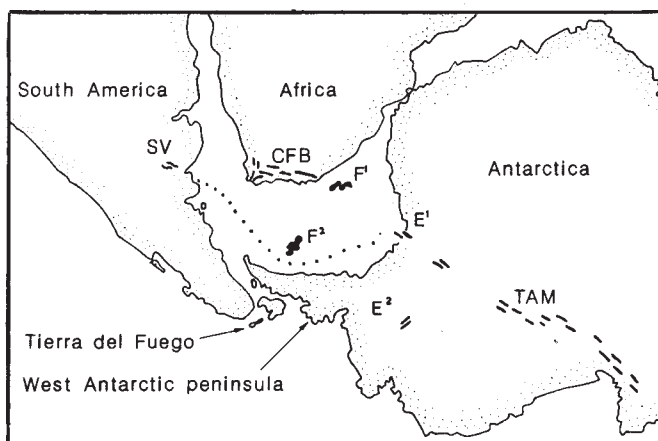
But the stable craton of Gondwanaland must include the 1,000-Myr-old Cape Meredith complex in the Falkland Islands. The Lower Cretaceous palaeoposition of the Falklands implies a south-westward bulge in the craton and the fore-arc terranes of Tierra del Fuego — 1,500 km from the Cape Fold Belt — is considered too distant for the two areas to be linked by subduction-related orogeny¹⁵, unless some extreme form of 'flat-plate' or low-angle subduction occurred. The rotation of the Falkland Islands to the eastern margin of South Africa removes the bulge in the craton, opening up a space in the Jurassic reconstruction (see figure) that may have been occupied by West Antarctic microplates. The distance between fore-arc terranes and the Cape Fold Belt may there-

fore be considerably reduced. The alignment of the fold vergences of the Falkland Plateau and the Ellsworth Block with the rest of the Gondwanide orogeny also weakens the argument¹⁴ that large variations in fold vergences are more typical of mobile belts within continental crust than subduction-related orogenies.

Although the rotation of the Falkland microplate solves some problems in south-western Gondwanaland, it raises a whole batch of new questions. How did the Falkland Islands move south-westward and rotate to their Lower Cretaceous palaeoposition? Was the movement associated with the mid-Jurassic–Cretaceous formation of the Outeniqua Basin that underlies the southern continental margin of South Africa and its apparent continuation in the central basin of the Falkland Plateau¹⁵? Is there some oceanic crust under the central Falkland Plateau¹⁶? In short, how was the Lower Cretaceous Falkland Plateau constituted?

There is evidence that some movement occurred between the South American mainland and Africa shortly before the South Atlantic opened. Pre-mid-Jurassic palaeomagnetic poles¹⁷ suggest that South America occupied a position about 300 km west of its palaeoposition next to Africa, as shown in the figure, a position almost identical to the one it occupied in the mid-Cretaceous after 10–12 Myr of seafloor spreading in the South Atlantic⁹. This suggests that there may have been separation between the two continents followed by closure before the Lower Cretaceous opening of the South Atlantic. Did such movements involve all of South America or was there relative movement between additional microplates within southern South America? Were these movements similar to or even associated with mid-Jurassic–lowermost Cretaceous (pre-Barremian) back-arc basins in Patagonia and Tierra del Fuego?¹⁸

All the microplates in the area have still to be identified and their palaeopositions solved. Moreover the configuration of the Patagonian and West Antarctic subduction zones and their relationship to Gondwanaland break-up is not yet known. The subduction of varied oceanic features could have led to the offset of spreading ridges around southern Africa by long



A new reconstruction of Gondwanaland¹⁹ showing the Lower Jurassic⁷ (F¹) and Lower Cretaceous⁹ (F²) palaeopositions of the Falkland Islands. Also shown are the rotated (E¹) and unrotated (E²) positions of the Ellsworth Block within Antarctica⁸. The dashes represent the Gondwanide Orogeny: SV, Sierra de la Ventana; CFB, Cape Fold Belt; TAM, Trans-Antarctic Mountains. The dotted line represents the southwestern bulge of the Gondwanide craton caused by the Falkland Islands in their Lower Cretaceous (F²) palaeoposition, which is removed if the rotated (F¹) position is adopted. Note that the West Antarctic peninsula is shown in its present day position.

transform faults, or complicated microplate tectonics and subduction could provide an explanation for the change in vergence between the Cedarberg and southern limbs of the Cape Fold Belt.

Answers may lie in new marine geophysical data from key areas of the southern ocean and in comprehensive palaeomagnetic, petrological, structural and geochronological studies of the various plates. The new work on the Falklands microplate shows that a different approach to an apparently simple reconstruction reveals a more complicated reality. □

- Harrison, C.G.A., Barron, E.J. & Hay, W.W. *Geology* **7**, 374 (1979).
- Veevers, J.J., Powell, C. McA. & Johnson, B.D. *Earth planet. Sci. Lett.* **51**, 435 (1980).
- Du Toit, A.L. *Our Wanderer Continents* (Oliver & Boyd, Edinburgh, 1937).
- Norton, I.O. & Sclater, J.G. *J. geophys. Res.* **84**, 6803 (1979).
- Dalziel, I.W.D. & Elliott, D.H. *Tectonics* **1**, 3 (1982).
- Watts, D.R. & Brumell, A.M. *Nature* **293**, 638 (1981).
- Mitchell, C., Taylor, G.K., Cox, K.G. & Shaw, J. *Nature* **319**, 131 (1986).
- Rabinowitz, P.D. & Labrecque, J.L. *J. geophys. Res.* **84**, 5973 (1979).
- Martin, A.K., Goodlad, S.W., Hartnady, C.J.H. & du Plessis, A. *Geophys. J. R. astr. Soc.* **71**, 567 (1982).
- Adie, R.J. *Geol. Mag.* **89**, 401 (1952).
- Dalziel, I.W.D. *Geology*, **8**, 260 (1980).
- Harrison, C.G.A., Barron, E.J. & Hay, W.W. *Geology*, **8**, 262 (1980).
- Dingle, R.V., Siesser, W.G. & Newton, A.R. *Mesozoic and Tertiary Geology of Southern Africa* (Balkema, Rotterdam, 1983).
- Halbich, I.W. in *Geodynamics of the Cape Fold Belt* (eds Sohng, A.P.G. & Halbich, I.W.) 177 (Geology Society of South Africa, 1983).
- Martin, A.K., Hartnady, C.J.H. & Goodlad, S.W. *Earth planet. Sci. Lett.* **54**, 293 (1981).
- Rabinowitz, P.D. *Geol. Soc. Am. Bull.* **91**, 655 (1980).
- Valencio, D.A., Vilas, J.F. & Pacca, I.G. *Geophys. J. R. astr. Soc.* **73**, 135 (1983).
- De Wit, M.J. & Stern, C.R. *Tectonophysics* **72**, 229 (1981).
- Martin, A.K. & Hartnady, C.J.H. *J. geophys. Res.* (in the press).

A.K. Martin is at the National Research Institute for Oceanology, PO Box 320, Stellenbosch 7600, South Africa.

Pauling's model not universally accepted

SIR—John Maddox¹ has announced that Linus Pauling “has once more thrown a cat among some pigeons”. This cat is a model of directed multiple twinning of a cubic crystal that Pauling² insists is the explanation of quasiperiodic crystals with icosahedral symmetry. Pauling's model is specific enough to be contradicted by many experimental observations from high resolution and dark-field transmissions electron microscopy, field ion microscopy, Mössbauer spectroscopy and various electron diffraction methods, most of which have been published.

For a twin model to be meaningful, the size of the individual crystals of the twin must be at least as large as the lattice parameter of 26.73 Å proposed by Pauling. The model is thereby trivially dismissed through a careful examination of the evidence already published of the high resolution (2 Å) transmission electron micrographs^{3–7} which show neither twins nor cubic unit cells. There are also detailed companion diffraction data from single icosahedral orientations akin to a conventional single-crystal pattern^{3–9}. Had Pauling taken the single crystal diffraction pattern of his predicted structure, rotated it into the 20 different orientations of his twinning model, and superimposed them, he would have seen immediately that his model fails to reproduce these observed diffraction patterns. Happily, contemporary crystallography has these exacting tools available to develop and verify proposed structures.

One of the most compelling observations to contradict the twinning hypothesis comes from field ion microscopy in which individual atoms are resolved¹⁰. The icosahedral symmetry is seen to extend over the entire field of view. Any twinning of crystals would have been observed and would have altered the symmetry.

From the beginning we viewed twinning as the obvious model within conventional crystallography^{8,9} for explaining our observations. Five-fold twins of the equilibrium orthorhombic Al₆Mn phase are occasionally seen in electron microscopy and easily identified as such (D. Shechtman, unpublished data and ref. 11). Micro-diffraction from areas containing equal amounts of all the twins gives a five-fold pattern, which is clearly indexable as coming from periodic crystals and which is different from that of the icosahedral phase. As predicted, dark-field imaging from different spots in turn highlights each twin separately. Micro-diffraction away from the twin boundaries clearly reveals single-crystal diffraction. However, for the icosahedral solid dark-field imaging from any spot highlights the entire grain⁹. With such evidence, sup-

plemented by convergent beam diffraction^{9,12}, twinning has, for some time, ceased to be a tenable hypothesis, even for small unit cells.

Pauling has nonetheless highlighted, however inadvertently, both the ongoing intense search for models of this structure and the basic questions this poses about crystallography. Crystal periodicity has been elevated from a nineteenth century hypothesis of crystallography to the status of a law or an axiom in spite of the well-known existence of incommensurately modulated crystals. In a thought experiment any finite structure can be reproduced and stacked in a periodic array. We could make a periodic model of a glass or of the icosahedral solid by taking a large enough cube, reproducing it exactly and stacking it. If the “unit cell” is large enough, this “periodic crystal” would be experimentally indistinguishable from a glass or the icosahedral solid. Such a large unit cell model would not need to be twinned as it contains internally the required symmetry approximately.

As an aside it should be noted that, because of defects, most conventional real crystals are not truly periodic, but crystallography ignores this and recognizes the ideal. Would it then matter if the artificial periodicity of the large unit cell models was also destroyed by defects?

We have in our own work¹³ used the cut and projection methods^{14–17} to generate in a systematic way a sequence of periodic structures of ever-increasing periods which in the limit become truly quasiperiodic. In such a sequence, the order and structural units are apparent on a scale which remains constant once the cell dimensions have become large — that is, as the cell dimension increases, the mesh of the diffraction pattern naturally becomes finer and the diffraction pattern deviates less and less from its quasiperiodic limit. Through these studies we can examine various categories of observable differences between the quasiperiodic phase and its long periodic approximant. This enables us to see which of these are the best indicators of large unit cells. The observations on the icosahedral phase are remarkably accurately fitted by the quasiperiodic limit. Pauling's model, and its attendant requirement for *post facto* twinning, simply does not fit anywhere in this sequence.

The *n*th member of the cubic approximants has a unit cell which is $4.8281\tau^n$ Å, where $\tau=1.618034=2\cos(\pi/5)$ is the golden mean which is an important geometric constant with 5-fold axes. Lattice constants of successive approximants increase by a factor of τ . Many d-spacings in the same direction are observed to increase with this ratio, for example, 1.2765 and 2.060 Å which Pauling incorrectly assigns nonparallel indexings of 20 6 2 and 10 8 2.

The fifth approximant is interesting for comparing with Pauling's model. Its lattice parameter is 53.545 Å, exactly twice Pauling's. In this cell the 2.168-Å d-spacing which is known to lie along the 5-fold axes has indexes 13 21 0, and for the m3 cubic point group has the 12-fold multiplicity required for icosahedral 5-fold axes. The exact 5-fold direction for icosahedral symmetry is $(1\ \tau\ 0)$. The ratio 21/13 of two successive Fibonacci numbers is an approximant to τ . The angles between the $(13\ 21\ 0)$ are close to what is required for icosahedral symmetry. There is no need to twin this structure to approximate icosahedral symmetry! On the other hand, Pauling assigns 12 2 2 to this spacing, that is, 24 4 4 on the doubled cell. It occurs in 24 different directions in just one of his 20 crystals. In his twinned structure the 480 spots with this d-spacing would occur in either 60 or 120 clusters depending on how much strain he allows. There are too many of them and they do not lie along the twelve 5-fold directions. Comparison with all the fifth approximant indexing shows that only the first two of Pauling's assignments are correct.

Because powder patterns are obtained from many crystals of all orientations, they do not reveal the rotational symmetries as graphically as do single-crystal patterns. By focusing on powder patterns alone, Pauling could attempt to ignore the 5-fold axes. But even the powder diffraction method reveals that many pairs of d-spacings are in ratio of the golden mean and more exact patterns would have required him to keep increasing his unit cell.

John Maddox¹ marvels at “how intuition, experience and apparently universal knowledge can be used to fashion a complicated and unknown crystal structure out of thin air”. Unfortunately, complicated crystal structures cannot be fashioned out of thin air. However, Pauling's stochastic method is justly famous when the proper building blocks are used. Pauling's basic building block of aluminium dodecahedra containing the transition element at its centre was wrong chemically. It has been ruled out by Mössbauer measurement on the icosahedral phase¹⁸. This building block does not occur in any of the known structures of periodic crystals of aluminium with manganese, or with manganese and silicon. The ternary combination may indeed contain packing units quite similar to those that occur in the icosahedral phase^{6,19}, and Pauling's method using these units may be more successful.

Experimental evidence is a convincing tool for ruling out models. There is by now a wide variety of experimental evidence to rule out both twinning of any sort and all structures of conventional periodic crystals except for those near the limit mentioned above. Pauling's model can only

claim a reasonable fit to the X-ray powder pattern data and is contradicted by everything else. The cat was dead. In spite of the commotion, the pigeons will endure.

JOHN W. CAHN

*Institute for Materials Science and Engineering,
National Bureau of Standards,
Gaithersburg, Maryland 20899, USA*

DENIS GRATIAS

*C.E.C.M./C.N.R.S.,
15 rue Georges Urbain,
94400 Vitry-sur-Seine, France*

DAN SHECHTMAN

*Department of Materials Engineering,
Israel Institute of Technology,
Technion, 32000 Haifa, Israel*

1. Maddox, J. *Nature* **317**, 417 (1985).
2. Pauling, L. *Nature* **317**, 512 (1985).
3. Hiraga, K., Hirabayashi, M., Inoue, A. & Masumoto, T. *Sci. Rep. RITU*, **A 32**, 309 (1985).
4. Bursill, L. & Lin, J. *Nature* **316**, 50 (1985).
5. Portier, R., Shechtman, D., Gratias, D. & Cahn, J.W. *J. Mic. Spectro. Elec.* **10**, 107 (1985).
6. Guyot, P. & Audier, M. *Phil. Mag.* **B 52**, L15 (1985).
7. Knowles, K.M., Greer, A.L., Saxton, W.O. & Stobbs, W.M. *Phil. Mag.* **B 52**, L31 (1985).
8. Shechtman, D., Blech, I., Gratias, D. & Cahn, J.W. *Phys. Rev. Lett.* **53**, 1951 (1984).
9. Shechtman, D. & Blech, I. *Met. Trans.* **16A**, 1005 (1985).
10. Melmed, A. & Klein, R. *Phys. Rev. Lett.* (submitted).
11. Zhang, Z., Ye, H.Q. & Kuo, K.H. preprint.
12. Bendersky, L. *Phys. Rev. Lett.* **55**, 1461 (1985).
13. Gratias, D. & Cahn, J.W. *Scripta Met.* (in the press).
14. Elser, V. *Phys. Rev. Lett.* **54**, 1730 (1985).
15. Elser, V. *An Introduction to Quasicrystal Diffraction*, preprint (1985), AT&T Bell Lab.
16. Duneau, M. & Katz, A. *Phys. Rev. Lett.* **54**, 2688 (1985).
17. Duneau, M. & Katz, A. *Quasiperiodic Patterns and Icosahedral Symmetry* preprint Ecole Polytechnique (France, 1985).
18. Swartzendruber, L., Shechtman, D., Bendersky, L. & Cahn, J.W. *Phys. Rev.* **B 32**, 1383 (1985).
19. Henley, C. & Elser, V. *Phys. Rev. Lett.* (in the press).

SIR—The icosahedral phases of alloys such as Al/Mn recently reported by Shechtman and others¹ and amply confirmed by several other groups, present many problems which are compounded by Pauling's demonstration² that the powder patterns can be explained as being due to a cubic structure (with $a = 26.74$ Å) which he postulates in some detail. I believe that some puzzling aspects of the situation can now be resolved.

There are two major viewpoints: (1) that the icosahedral phase is a materialization of the Penrose pattern and (2) Pauling's view, that the phase is a result of the twinning of cubic crystallites. I sketch here my reasons for preferring the former.

Normal crystal phases fall into one or other of the 230 space groups. Their diffraction effects have the three-dimensional point symmetries which are one or other of the 32 crystallographic point groups. The diffraction symmetry of the icosahedral phases is $m\bar{3}5$, which is not one of the 32 crystallographic point groups and thus the real space structure giving rise to the diffraction cannot be that of a normal crystal. What then is it? I assert that it is a particularly fine-grained and highly ordered texture corresponding to what might be called an 'icosahedral

coincidence site lattice'. This might also be seen³ as an icosahedral nematic, again perhaps the most ordered possible.

High resolution electron micrographs produced by several groups show no signs of the twin boundaries which would be necessary for Pauling's model. Twinning must therefore be at a near to atomic level.

The basic physical observation⁴ is that the diffraction patterns show that there are three strong coplanar reciprocal lattice vectors (corresponding to planes of spacings 2.168, 2.060, 1.2765 Å) which lie in the directions of the 5-fold, 2-fold, 2-fold axes of a dodecahedron, respectively, and that the first is half the vector sum of the others. The indices (12,2,2), (10,8,2) and (20,6,2), allocated by Pauling, are incompatible with this. Further, Pauling allocates to strong reflections in the ratio of $1:\tau:\tau^2$ the cubic cell indices with $n = 64$, 168 and 440 which are close to the squares of 8, 13 and 21 from the Fibonacci series, but he neglects the angular relationships between the strong reflections. (Note: τ is the golden number $(1 + \sqrt{5})/2$.)

The effect of strong structure factors for planes, which would be the $(\tau, 1, 0)$ planes in a cubic cell (parallel to the faces of a regular dodecahedron), is to produce two periodicities, in the incommensurable ratio of τ , along the 2-fold axes. The published electron micrographs clearly show this irregularity. From this, everything else flows. In order to have a material structure with two such overlapping periodicities there must be a determinate or statistical rule to decide which has precedence at a particular site. The Penrose tiling is such a determinate rule.

The Penrose tiling gives a convincing indexing of the diffraction patterns^{4,5}, but leaves open the problem of placing atoms in the quasi-unit-cells, for which various suggestions have been made.

The essential geometry is to be found in the demonstration by Euclid that five cubes may be inscribed in a regular dodecahedron. Pauling emphasizes the cubic nature of the twinning, but gives no guidance as to how it may be continued indefinitely. The quasi-crystal view emphasizes the icosahedral quasi-lattice and provides an algorithm for indefinitely great extension.

The twin relationship arising from seeing five cubes inscribed in a dodecahedron, means that along the 5-fold axis all five cubes are viewed in the $[\tau, 1, 0]$ direction. In the three-fold view, two cubes (related as a $\Sigma = 3$ coincidence site lattice) are viewed along $[111]$ and three along $[(1 + \tau), 1, 0]$ and in the 2-fold direction $[100]$ for one cube and $[\tau, 1, 1/\tau]$ for four cubes. We may ask how the five structures intersect as a finite grouping and how such an arrangement may be extended.

The diffraction effects of the ico-

sahedral phase thus resemble those of a twinned cubic texture, of a Penrose tiling and of an incommensurate structure. A quasi-lattice in N -dimensions was originally defined⁶ as one where lattice points are arranged with integral coordinates with respect to more than N axes. The hexagonal Miller-Bravais system is a familiar case, but if the axial lattice vectors are incommensurate with each other, then incommensurate periods in the same direction can occur.

We assert that the Penrose tiling can be regarded as: (i) an icosahedral nematic structure, (ii) an icosahedral coincidence site lattice and (iii) a generator of homometric structures. Two structures are homometric if they have the same diffraction pattern but different structures (spatial arrangements of the scattering matter). Thus they have the same set of amplitudes for the Fourier terms but different sets of phases. (We wish to point out that these sets of phases may have symmetries different from that of the point group of the diffraction amplitudes.)

If we take threads along the 2-fold axes then the Penrose tiling may be covered (three times over) by the 15 threads, each rhombohedron being crossed by three threads, each entering through one face and leaving through the face opposite. From this it follows that the spacing of the planes of tiles with a common edge is twice the unit rhombohedral edge. In the Penrose tiling these are planes across the structure parallel to the faces of a regular dodecahedron and at these uniform intervals. The strongest diffraction line corresponds to a spacing of 2.170 Å for these planes. With the indices of Bancel *et al.*⁴, this strongest plane would correspond to the layers of tiles, but the edge of the fundamental rhombohedra would be only 1.085 Å. They could thus occur only statistically. On Elser's indexing⁵ the fundamental edge would be τ^3 times this, namely 4.60 Å, but the layers would then be 9.20 Å apart and it is then necessary to explain why the strongest Bragg planes have a spacing of $1/\tau^3$ times this.

The nature of the threads remains open but they may be imagined, for example, as icosahedra (of Al around Mn) sharing edges. The Penrose pattern would describe how one set of threads can pass through another.

In view of the production of the icosahedral phase by rapid cooling it is unlikely that the potentiality of the Penrose pattern to prescribe an exactly determinate structure of infinite extent is used. The problem is the classical one of packing dodecahedra which almost fit to fill space.

The diffraction properties of a quasi-lattice are subtle and unexpected. The Fourier transform of an extended one-dimensional Fibonacci series of long and short intervals (in the ratio of τ) is the same as the series itself. This is also the

case in more than one dimension, and holds for those arrays of lattice points which can be derived by bounded projection from a lattice of higher dimensionality (an alternative definition of a quasilattice)⁵. Determination of the atomic arrangement still, as far as we know, waits for the development of a method of calculating structure factors and for the apportionment of statistical and determinate features of the texture.

ALAN L. MACKAY

*Department of Crystallography,
Birkbeck College,
University of London,
Malet Street, London WC1E 7HX, UK*

1. Shechtman, D., Blech, I., Gratias, D. & Cahn, J.W. *Phys. Rev. Lett.* **53**, 1951 (1984).
2. Pauling, L. *Nature* **317**, 512–514 (1985).
3. Nelson, D. & Sachdev, S. *Phys. Rev.* **B32**, 689–695.
4. Bancel, P.A., Heiney, P.A., Stephens, P.W., Goldman, A.I. & Horn, P.M. *Phys. Rev. Lett.* **54**, 2422–2425 (1985).
5. Elser, V. *Acta crystallogr.* (in the press).
6. Mackay, A.L. *Sov. Phys. Cryst.* **26**, 517–522 (1981) *Physica* **114A**, 609–613 (1982).

SIR—We recently presented X-ray and electron diffraction data on the icosahedral phase of rapidly quenched Al–Mn and introduced a simple indexing scheme based on the symmetry of an icosahedron (Bancel *et al.*, ref. 1 and in the press). We used our X-ray diffraction measurements to argue against models for the structure of this material that require multiple scattering from different crystallites to explain the electron diffraction patterns. Linus Pauling has proposed² a new crystalline model for the icosahedral phase, which he compares with X-ray powder diffraction data obtained from Shechtman and Blech³.

In Pauling's model, the icosahedral symmetry observed in the electron diffraction patterns is accommodated by multiple twinning of cubic crystals. Although twinning makes the analysis more complicated, it is still possible to check whether the (hkl) indices given in Table 1 of Pauling's paper can be consistent with the observed electron diffraction patterns. Consider any two of Pauling's cubic vectors, which are separated by some angle ϕ . Upon twinning, each vector is rotated via a set of icosahedral symmetry operations to a new set of positions. The same twinning operations act upon both cubic vectors, so that the angle ϕ is preserved during rotation. If one now calculates the angle that the vectors of one icosahedral set make with the vectors of the other set, the angle ϕ should certainly appear. Pauling's model fails this test on nearly all counts¹. Thus, the icosahedral diffraction spots cannot be attributed to rotations of the cubic structure which Pauling proposes.

Pauling states that the agreement between the X-ray peak positions of Shechtman's data and the d-spacings predicted by his model provides compelling evi-

dence that his structure is the correct one. We are not convinced by this argument because we believe that seven of the peaks he indexes are actually due to a different phase present in the sample. We have measured X-ray powder diffraction patterns from a large number of rapidly quenched icosahedral alloys, and find that reflections 3,4,6,8,10,15, and 16 listed in Pauling's Table are due to a contaminant phase that appears when excess Si is present in the sample. These peaks are not found in the electron diffraction patterns. (Note that Shechtman's sample contained several atomic % Si). We feel that this fortuitous agreement of several spurious peaks is an argument for the hypothesis that any powder diffraction pattern can be indexed via a sufficiently large cubic unit cell.

The question remains as to whether a crystal structure incorporating a large unit cell might still describe the electron and X-ray diffraction data. Based on recent low angle electron and X-ray diffraction data we can specify a minimum size for any proposed cubic unit cell. For any periodic structure the squares of any two d-spacings must be in integer ratios unless the corresponding diffraction spots have the same symmetry (so are collinear) in which case the ratio of the d-spacings is a ratio of integers. We have observed two such spots which lie along the major axes of the 2-, 3-, and 5-fold symmetry planes with d-spacings of 8.85(7) and 5.42(3) Å, the ratio of which is 1.633. The lowest rational approximate, within experimental error, to this number, is 13/8. This implies a minimum cell size of $a_0 = 8(8.85 \text{ Å}) = 71 \text{ Å}$. A 27 Å cubic unit cell cannot produce two *collinear* spots with these radii even with the inclusion of multiple twinning. Although our arguments are stated in the context of a twinning model, numerous high resolution microscopy measurements vitiate the possibility of twins in these structures. In the absence of twinning our limit of 70 Å as a minimum cell size holds and is valid for any crystal symmetry. A crystal structure adhering to this criterion would have to include well over 10,000 atoms in its unit cell.

Finally, we find somewhat curious Pauling's statement that "Crystallographers can now cease to worry that the validity of one of the accepted bases of their science has been questioned". No one has seriously questioned the premise that fivefold or icosahedral symmetry is inconsistent with periodic translational order. Rather, it has been observed that particular arrangements of atoms without simple periodic translational order and with crystallographically disallowed symmetries may nonetheless give rise to sharp diffraction patterns⁴. The study of "quasicrystalline" structures is mathematically intriguing. Furthermore, even if all the phases of Al–Mn should turn out to be crystalline, the

theory of quasicrystalline materials may well provide clues to the properties of materials that have local icosahedral symmetry.

PETER A. BANCEL

PAUL A. HEINEY

*Department of Physics and Laboratory
for Research on the Study of Matter,
University of Pennsylvania,
Philadelphia, Pennsylvania 19104, USA*

PETER W. STEPHENS

*Department of Physics,
State University of New York,
Stony Brook, New York 11794, USA*

ALAN I. GOLDMAN

*Physics Department,
Brookhaven National Laboratory,
Upton, New York 11973, USA*

1. Bancel, P.A., Heiney, P.A., Stephens, P.W., Goldman, A.I. & Horn, P.M. *Phys. Rev. Lett.* **54**, 2422 (1985).
2. Pauling, L. *Nature* **317**, 512 (1985).
3. Shechtman, D. & Blech, I. *Metal Trans.* **16A**, 1005 (1985).
4. Levine, D. & Steinhardt, P.J. *Phys. Rev. Lett.* **53**, 2477 (1984).

SIR—In the analysis of apparent icosahedral symmetry of Al–(Mn, Fe) alloys by Pauling¹ one observation remained unaccounted for. Mössbauer data of Swartzendruber *et al.*² indicate the presence of two type of sites for the transition metal. The occurrence ratio was found to be close to the golden mean ($(\sqrt{5}+1)/2=1.61803$). In the model of an aperiodic "icosahedral phase" this can be easily explained by two types of differently distorted icosahedra. In order to fill the three-dimensional space in the sense of the 5-fold Penrose tile the occurrence ratio of two different elements should be exactly the golden mean.

On the other hand, in the Pauling's picture of multiple twinning of cubic crystal the growth process can be seen as starting from the 12-vertex icosahedral seed (Fig. 1 of ref. 3), with the next layer being dodecahedral (20 atoms). In any structure based on these two mutually complementary polyhedra the occurrence ratio of 20/12 = 1.667 could be expected for different types of lattice sites. But this ratio differs from the golden mean by only 3%.

A plausible suggestion is, therefore, that Swartzendruber *et al.*² did, in fact, observe the above ratio (20/12) rather than the golden mean. If this is the case, then the dilemma between the "icosahedral phase" and Pauling's model based on a cubic cell could be resolved by the Mössbauer experiment with the peak intensity resolution greater than 3%.

A.A. BEREZIN

*Department of Engineering Physics,
McMaster University,
Hamilton, Ontario,
Canada L8S 4M1*

1. Pauling, L. *Nature* **317**, 512 (1985).
2. Swartzendruber, L.J., Shechtman, D., Bendersky, L. & Cahn, J.W. *Phys. Rev. B* **32**, 1383 (1985).
3. Bergman, G., Waugh, J.L.T. & Pauling, L. *Acta crystallogr.* **10**, 254 (1957).

Taking culture seriously

Philip Kitcher

Culture and the Evolutionary Process. By Robert Boyd and Peter J. Richerson.
Chicago University Press: 1985. Pp.331. \$30, £25.50.

FOR true believers in biology as the key to understanding human behaviour, "culture" is a word that cowards use. Sociobiological zealots maintain that the invocation of culture as an evolutionary force obfuscates Darwin's insight that human behaviour is the product of evolution. At the other extreme are those who refuse to commit social science, protesting that the combined efforts of anthropologists, sociologists and mislocated biologists teach us far less about human nature and human conduct than the observations of a Shakespeare or a Stendhal. Human culture, they declare, is too complex for analysis, and it radically transforms the simple forms of behaviour we have inherited from hominid or prehomid ancestors. Between the militant sociobiologists and the despondent sceptics lies a large territory, explored by a small number of scholars who hope to take human culture seriously and to analyse its evolutionary effects. Robert Boyd and Peter Richerson have charted some of this territory with exemplary skill and determination.

Culture and the Evolutionary Process addresses two general problems. Boyd and Richerson want to understand the evolutionary effects of various systems of cultural transmission, conceived as systems in which individuals may acquire phenotypic characteristics at least partly as a result of imitating any number of cultural "parents" or "models". They attempt to identify the various forces that can preserve or change the distribution of traits across the generations of a population having a system of cultural transmission. Their second general question concerns the possibility of maintaining systems of cultural transmission under ordinary selection.

The second question arises because Boyd and Richerson see their book as a (polite) debate with conventional human sociobiologists. (In my view, the deference is overdone and the differences understated.) If it were shown merely that certain conditions of cultural transmission might lead members of a population to forms of behaviour distinct from the sociobiological optima, then there would be a simple retort: where cultural transmission proves maladaptive, selection will work to eliminate it; hence culture can only be maintained when it helps to amass evolutionary capital; we may fathom human behaviour by looking at reproductive success and forgetting culture.

The book tackles its main questions by mathematical analyses and discursive reviews of findings from psychology, anthropology and sociology. The mathematics is intended to pinpoint the conditions under which various kinds of evolutionary processes can occur. In particular, the authors want to specify the circumstances that would allow for characteristics detracting from genetic fitness to become prevalent. Their surveys of the social sciences are to provide motivation for the mathematical models they construct and to suggest that it is possible, even likely, that the conditions for the maintenance of genetically maladaptive behaviour may be met.

Inspired by Lewontin's well-known account of the structure of population genetics, Boyd and Richerson begin with a representation of an idealized life-cycle in which genetic and cultural transmission jointly affect the distributions of genotypes and phenotypes in descendant generations (pp.5–6). The representation raises at once the obvious possibilities that the book will explore. Does the existence of more than two cultural "parents" make a difference? What are the features of blending and non-blending cultural inheritance? How does imitation interact with individual learning? What happens if the traits that make someone culturally influential differ from those that promote reproductive success? Should we expect that people will be more likely to be influenced by those who have fitness-enhancing characteristics? When will it pay to follow the majority?

The authors develop their basic account of cultural transmission by simplifying ideas worked out in great detail by Cavalli-Sforza and Feldman. Imagine that people are "enculturated" by a fixed number of individuals playing different roles, and that each role has a weight in the transmission of a characteristic. The occupant of each role may have or lack the characteristic. Consider any possible combination. There will be a probability that a person "enculturated" by that sequence of cultural parents will acquire the characteristic. The system of cultural transmission may be identified by specifying the probabilities for all the possible combinations. As the authors note, this approach to systems of cultural transmission allows them to represent both blending and non-blending inheritance.

Analogues of genetic mutation and drift

are easily introduced into the basic picture (pp.67–69). But the interesting disruptive forces are those to which Boyd and Richerson devote most attention. The first involves a contrast between individual and social learning. Imagine that the young acquire their adult behaviour by striking a compromise between imitation and individual attempts to learn. If cultural transmission operated unconstrained it would lead them to adopt a certain value of a behavioural parameter; their own efforts lead them to an estimate (possibly error-infected) of the optimal value of the parameter; the adult behaviour expresses a weighted average of the two. Boyd and Richerson show that this scenario involves a force that will increase the frequency of traits favoured by learning, a force they call "guided variation" (pp.95–97). Since the effect of guided variation is adaptive when the individuals are good at determining the best value of the behavioural parameter, it is natural to ask if natural selection will allow for a non-zero weight to be assigned to cultural transmission. The authors give a rigorous demonstration of the intuitive idea that errors in learning and costs of learning may outweigh possible benefits of distrusting tradition. Sometimes the wise child should agree that teachers know best.

Just as guided variation can mimic natural selection, so too can another force that Boyd and Richerson discuss at length. Suppose there is genetic variation with respect to the propensity for imitating traits that lead to reproductive success. Then we can expect selection to favour alleles whose bearers follow the fit. As the authors put it, if guided variation and direct bias were the only forces that shape cultural evolution, then "... with the usual caveats, we would be able to predict cultural variation by asking what increases genetic fitness" (p.157).

But it is the power of other forces that is the central theme of the story. Boyd and Richerson propose three ways in which culture can favour the genetically maladaptive. Individuals may base their behaviour on the conduct of those around them. If this occurs, the authors suggest, there may be an analogue of group selection which allows for the evolution of cooperative arrangements. Second, there may be indirect bias, in which some forms of behaviour give a person high status as a model, with the result that other, correlated, traits are also imitated. (Intuitively, the process is analogous to genetic linkage.) Boyd and Richerson offer an analysis of indirect bias, arguing that it can have effects like those of runaway sexual selection. The third force is the simplest. Transmission systems are said to be "asymmetric" if they favour different traits. If the characteristics that make one a successful cultural "parent" are incom-

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

©Anglo-Australian Telescope Board

Edge of darkness: the aptly named Sombrero Galaxy, in Virgo (the "brim" is the dust lanes of the galaxy's central plane). The photograph is reproduced from Peter Cadogan's From Quark to Quasar: Notes on the Scale of the Universe, recently published by Cambridge University Press, a highly illustrated journey from the human scale to the very large and the very small. Price of the book is £12.95, \$24.95.

patible with some that promote reproductive success, then it is possible for culture to disrupt the smooth workings of natural selection.

The main thrust of *Culture and the Evolutionary Process* can be appreciated by focusing on this last force. Boyd and Richerson construct a sequence of analyses (purchasing decreases in artificiality with increasing complexity) in which they identify precise conditions for the maintenance of a maladaptive trait. As we might expect, selection against the trait has to be relatively weak and the competition for dominant cultural role relatively intense. (The authors tell us *how* weak and *how* intense.) Now comes the sociobiologists' challenge: if cultural transmission is asymmetric with respect to genetic transmission, will selection eliminate it in favour of a system that will reward reproductive success? Not necessarily, claim Boyd and Richerson. For there may be indirect constraints that force certain reproductively successful individuals out of the competition for cultural influence, as well as direct benefits deriving from "enculturation" by a large sample of the local population (pp.188–190).

The authors sum up in a typically modest and cautious way. After noting that their analyses "suggest that asymmetric cultural transmission may cause human evolution to diverge from the predictions of sociobiological theory", they add: "Whether and to what extent it does is an empirical problem" (p.199). More self-congratulatory writers might have seen themselves as constructing a framework within which alternative hypotheses about human behaviour and its evolution could at last be formulated and tested against one another. For what Boyd and Richerson do is to show that there are far more things in the evolutionary process than are dreamed of by most sociobiologists.

Perhaps the chief danger is not that this book will languish unread but that it will

serve as the gospel for a new social scientific creed. Reformed sociobiologists may confute their sceptical opponents by announcing that Boyd and Richerson have solved the problem of culture. But the authors themselves claim far less, and rightly so, for there are important questions on which they touch either scantily or not at all.

Consider the focus on cultural transmission. Quite early on, Boyd and Richerson commit themselves to the idea that "culture can profitably be viewed as a system of inheritance" (p.19). But is the entire effect of culture on human evolution to be represented in terms of the transmission of traits that individuals can acquire or reject? Many social institutions are hard to represent in such terms (think of apartheid). Moreover, such institutions frequently have the effect of assigning the members of certain subpopulations to radically different microenvironments with large consequences both for the phenotypes that develop and for the determinants of fitness (again, think of apartheid). Boyd and Richerson step gingerly around the controversy about holism and reductionism without explaining how they plan to treat features of human society such as these.

A second limitation concerns the issue of linkage among cultural traits. One of the banal facts of life is that choices constrain. Certain forms of behaviour preclude others. Moreover, the choices that a person makes are affected both by the actual constraints and those that are perceived to exist. Once these truisms are given their due, we see that there is a type of linkage, possibly important, besides that captured in Boyd and Richerson's indirect bias. To adapt the analogy, the course of evolution at one cultural "locus" may have effects on what happens elsewhere. Further, the ability to free oneself from some constraints might itself be shaped by selection. Perhaps there are

adaptive advantages in being able to perceive certain logical and probabilistic connections.

Culture and the Evolutionary Process inherits from traditional sociobiology the habit of treating particular pieces of human behaviour as if they were individually shaped by the evolutionary process. In presenting new mathematical ideas this may simply be pardonable simplification (although the discussion of challenges to adaptationism on pp.282–283 is uncharacteristically superficial). Nevertheless, as social scientists consider the possibility of applying these ideas to aspects of human behaviour, it is crucial to appreciate the possibility that the proximate mechanisms of a piece of behaviour may be implicated in many areas of our social lives. Informative recent studies in descriptive anthropology have sometimes been accompanied by a superfluous and irritating descendant to the effect that minute details of behaviour enhance reproductive success. It would be sad if Boyd and Richerson's pioneering efforts spawned a mindless pseudo-adaptationist programme, in which developmental and proximate constraints were ignored and the optima of the new mathematical analyses perceived everywhere.

This book does not iron out a minor wrinkle in the biology of human social behaviour. Rather it addresses one major part of an enormous problem, and it does so with erudition, rigour and imagination. *Culture and the Evolutionary Process* is not easy going, but it deserves a broad readership and a lasting influence. With luck we may one day transcend the dialogue between simplistic reductionism and despairing scepticism. □

Philip Kitcher is Director of the Minnesota Center for the Philosophy of Science, University of Minnesota, Minneapolis, Minnesota 55455, USA. His most recent book is Vaulting Ambition: Sociobiology and the Quest for Human Nature (MIT Press, 1985).

More than perceptive

P.W. Hawkes

Pattern Recognition: Human and Mechanical. By Satoshi Watanabe. Wiley: 1985. Pp.570. \$44.95, £46.

PATTERN recognition has always been an uneasy subject. There is no obvious definition of "pattern" to fit everything that is grouped within the subject — letters and numbers and the characters of such languages as Chinese and Japanese, chromosomes, ship or aircraft profiles, types of terrain as seen from above, human cell categories, the list is as long as it is varied. It is even more difficult to tie down "recognition", for the idea is closely linked to the human faculty of recognition; the latter is so extraordinary that any attempt to emulate or simulate it at other than a trivial level seems inconceivable. Typists perform stupefying feats of pattern recognition on hand-written material; and once one begins really to think about the problems, it seems that the schoolteacher is performing a much more formidable task in recognizing his new intake of pupils each year than in acquiring his diplomas and degrees.

These difficulties have meant that, unlike most subjects of a primarily mathematical nature, pattern recognition has generated a considerable literature of a philosophical kind, devoted not so much to methods of performing pattern recognition tasks by computer as to understanding what such tasks involve, indeed, to understanding what the brain is doing as it tries to perform them. In this large and difficult book, Professor Watanabe has gone more deeply than most into such questions.

The volume is dedicated "To Dorothea for her patience and cheerful encouragement" and the reader may well sigh for a similar companion for the text is very difficult going, largely because of the language employed: it is inevitable that a young and complicated subject will need some new vocabulary, but it is not necessary to adopt the habits of the least literate kind of sociological writing. All too often Professor Watanabe falls into this style, with the result that some passages are so (linguistically) opaque as to be almost impenetrable. This is sad because, even if obscured by the language in which they are conveyed, the ideas brought together here deserve to be pondered carefully by practitioners of the subject. We are led through a number of ways of regarding the notion of pattern, with a host of down-to-earth examples, ranging from the conceptual (pattern recognition as seeing-one-in-many, as perception, as value-orientated ponderation) to the more overtly mathematical (pattern recognition as entropy

minimization, as covariance diagonalization, as discrimination, as structure analysis).

Watanabe expects his readers to work hard, for they must not be daunted by encounters with sensory physiology, classical philosophy or thermodynamic reasoning; they must be at ease with the formidable notation of formal logic; and they must be happy with linear algebra and statistical decision theory. The author does try very hard to explain everything as he goes along, but the more the reader knows beforehand of the tools employed, the more benefit he will gain from the text and the greater will be his admiration for the way the numerous threads are woven together to produce — the metaphor is

well-deserved — a very impressive pattern.

This, then, is a rich and densely filled book, occasionally verbose and unconsciously elliptical but exciting and definitely worth the effort demanded in reading it. Pattern recognition will presumably be tamed over the decades as our understanding of the mathematical and cognitive processes involved advances. I suspect that readers who then turn back to their precursors' books will be impressed by the ability of Watanabe to see where the problems lie and to shed some light on their nature at so primitive a stage of the subject. □

P.W. Hawkes is *Maître de Recherche* at the *Laboratoire d'Optique Electronique du CNRS*, BP 4347, F-31055 Toulouse Cedex, France.

Developing concepts

Raymond D. Lund

Molecular Bases of Neural Development. Edited by Gerald M. Edelman, W. Einar Gall and W. Maxwell Cowan. Wiley: 1985. Pp.606. \$85, £87.50.

RESEARCH on the development of the nervous system has for many years been constrained by techniques which are largely inadequate, especially when compared with the richness of theories offered by early generations of scientists. Now, however, we have the exciting opportunity to define and sequence antigens of developmental significance, to collect cDNA libraries and to study the molecules responsible for assembling the extraordinary structures which make up the nervous system.

There was, therefore, good reason for the Neurosciences Institute of New York, a leading body in the world of the neurosciences, to assemble a symposium around this new research initiative, and to prepare this, its fourth major publication since its inception in 1981.

The book is divided into six sections concerning early development, glia and neurone guidance, neural crest, formation of neurites and synapses, maps and retinotectal system and, finally, gene expression, "primary processes" and behaviour. An elegant introduction by Cowan summarizes the history of work on neural development, and in the last few sentences emphasizes the excitement ahead as this field increasingly becomes the province of molecular biologists.

The introduction is followed by a well-written set of essays by some of the most distinguished investigators currently working in this area. A wide variety of material is covered. For example, given the new insight provided by studies of the adhesion molecule, N-CAM, Rakic has re-examined the field of cerebellar development in

which he was heavily involved some years ago. Many articles such as those of Edelman, Letourneau, Goodman, Gershon and Schmidt review the current thrust of research in their laboratories. Yet others have reviewed fields to which they have contributed a smaller part. The diversity of approaches and the content of many of the articles make for interesting reading. None of them is dull and most are easily accessible to readers with only a limited background in developmental neurobiology.

As with all volumes which arise from a meeting, the book cannot hope to be comprehensive. However, one wonders, despite the justifications offered, why a fifth of it is devoted to the retinotectal system, a topic that has perhaps suffered from overexposure (and one ironically in which the developmentally significant molecules suggested over 40 years ago still elude identification). Surprisingly, little space is devoted to areas such as transmitter modulation, growth factors, acetylcholine receptor ontogeny, development of cell identity and genetic controls of development.

Indeed the amount of space devoted to molecular biology is quite small. This is in marked contrast to the Cold Spring Harbor Symposium of 1983 on molecular neurobiology. It is a pity also that no one thought to write a summary to balance Cowan's introduction, outlining how our present state of knowledge might be extended and how the approaches presented at the symposium might develop in the light of the recent technical and conceptual advances.

To summarize, it must have been a marvellous symposium and the book presents an elegant set of essays on neural development — among the best of their kind — but it only rarely enlightens on the molecular bases of development. □

Raymond D. Lund is in the Department of Anatomy and Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA.

Cycling back to the beginning

Michael Whitfield

The Carbon Cycle and Atmospheric CO₂: Natural Variations Archean to Present. Edited by E.T. Sundquist and W.S. Broecker. *American Geophysical Union*: 1985. Pp.627. \$28.

It is refreshing — although somewhat disconcerting — for someone involved in the investigation of the contemporary global CO₂ cycle and its possible climatic effects to view the problem from a much broader perspective. This volume jolts the reader into considering such a wider view by presenting a stimulating potpourri of ideas on the direction, cause and consequences of natural fluctuations in atmospheric CO₂ levels over the past 3.5×10^9 yr.

There are more than 40 papers, replete with useful data and providing intriguing insights into the response of different feedback systems to perturbations in the global carbon cycle over timescales varying from tens of thousands to hundreds of millions of years. The papers themselves have been edited thoroughly, and the text is clearly presented and well illustrated. The overall structure, too, is well conceived, in that the contents list is organized into five sections which move progressively further back in time. The first section is intended to link present-day concerns to the geological perspective while subsequent sections take the reader in turn through the last glaciation, the Pleistocene, the Cenozoic, and finally the Phanerozoic and Precambrian. There is a wealth of information here which, however, is unfortunately difficult to dig out because the book has no index and the papers within each section do not necessarily follow a logical sequence. Furthermore the sections identified in the contents list are not explicitly identified in the text, so that the browser finds himself slipping unwittingly from one timescale to another.

The first three sections cover the past 5×10^5 yr and mainly deal with the use of the carbon and oxygen isotope records in ice cores and in deep-sea sediments to elucidate the role of the oceans in controlling fluctuations in atmospheric CO₂ levels. Only one paper deals with the influence of the terrestrial biota on carbon storage. There are many absorbing accounts here of the potential and pitfalls of isotopic techniques for estimating atmospheric CO₂ levels, ice volumes, palaeoproductivity and palaeocirculation patterns. Over this timescale, orbital variations appear to trigger changes in atmospheric CO₂ levels which are, in turn, followed by changes in ice volume.

During the Cenozoic (10^8 yr), processes

such as volcanism, tectonic movement, continental weathering and organic carbon burial begin to assume a dominant role. In addition to isotopic clues, crises in the biological record characterized by mass extinctions provide useful markers against which to assess the interaction between the biota and the atmosphere. Surprisingly, only the Cretaceous/Tertiary event is considered here, although the discussion is spiced by the presentation of two conflicting hypotheses (planetismal impact and mantle outgassing).

Geochemical models for CO₂ control in the Phanerozoic are covered in some detail in the final section, but the treatment of the Precambrian is rather sketchy so that only 15 per cent of the book as a whole considers the first 99.75 per cent of geological time! The overall picture suggested is of a punctuated fall from atmospheric CO₂ concentrations one hundred

times the present level 3×10^9 yr ago, through a value approximately ten times the present level 10^8 yr ago, to a pre-industrial value only 80 per cent of the present level. The burial and preservation of organic carbon has played a primary role in this decline — a factor which geochemical modellers are now taking into account.

This is a fascinating collection of papers which will repay the effort required for specialist readers to pick their way through such a variety of information. The cost is very reasonable, and one can hope that many individuals will buy the book and use it to help them place the current ferment in atmospheric CO₂ research into a proper perspective. □

Michael Whitfield is a Senior Principal Scientific Officer at the Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK.

Uncertain alliance

Charles Tanford

The Physical Chemistry of Membranes: An Introduction to the Structure and Dynamics of Biological Membranes. By Brian L. Silver. *Allen & Unwin*: 1985. Pp.396. £45, \$60.

To present a concise account of all physico-chemical aspects of membranes in a single book, suitable for students and beginning researchers, is difficult because membrane physical chemistry comprises two quite different bodies of knowledge. One centres on the structure and molecular dynamics of the membrane, and includes the physical methods (nuclear magnetic resonance, neutron scattering etc.) used for structural investigations but should also include some account of the molecular interactions that hold the membrane together. The other part centres on the physical chemistry of membrane function, and is chiefly thermodynamic and kinetic in its approach to questions of membrane potential, permeability, and carrier or protein mediated transport. Conventional wisdom suggests that these two fields might be better tackled by separate authors in separate books.

Dr Silver's book attempts to include all these topics but regrettably cannot be recommended for either structural or functional information. Its broad organization is good: 18 chapters with appropriate titles. Within each chapter, unfortunately, there is insufficiency, often chaos, sometimes error, and always a flip-pant haphazardness as to what is selected for discussion and what is omitted, who is cited and who is not. The entire book has only 378 pages of text which includes 125 figures, many of them full page.

The better chapters are one that deals with order and disorder, as seen by nuclear magnetic resonance and electron spin resonance; one on intermolecular forces, which includes a mathematically detailed section on van der Waals' forces; and one on fluctuations, which includes an introduction to stochastic processes. Order/disorder is in part explained by picturesque images (a box of uncooked spaghetti, a pile of sleepy earthworms) that may be helpful to some students.

The poorer chapters are those that deal with solution physical chemistry (thermodynamics, kinetics, diffusion, membrane potential etc.). For example, the "celebrated Nernst equilibrium equation" is derived, properly limited to boundaries with the same solvent on both sides, only to be applied on the very next page to the equilibrium distribution of an ion between the *membrane interior* and the adjacent solution.

In the next chapter, on passive transport, the author argues as to whether or not diffusion can be regarded as arising from the action of a force. His conclusion is not clear but a subsequent parenthetical statement — "The theoretically inclined will perhaps remember that the diffusion coefficient is related to the autocorrelation function for the random force beating on the molecule" — implies perhaps that diffusion can be regarded in this way. The following chapter is centred on Eyring's absolute rate theory for diffusion, but misrepresents the basic ideas behind the theory by confusing energy and free energy of the activated state.

Silver has a lively style and an instinct for apt imagery. Combination of these assets with more careful theoretical analysis could produce a truly useful book. □

Charles Tanford is J.B. Duke Professor of Physiology at Duke University, Box 3709-P, Durham, North Carolina 27710, USA.

Future global warming from atmospheric trace gases

Robert E. Dickinson & Ralph J. Cicerone

National Center for Atmospheric Research, Boulder, Colorado 80307, USA

Human activity this century has increased the concentrations of atmospheric trace gases, which in turn has elevated global surface temperatures by blocking the escape of thermal infrared radiation. Natural climate variations are masking this temperature increase, but further additions of trace gases during the next 65 years could double or even quadruple the present effects, causing the global average temperature to rise by at least 1 °C and possibly by more than 5 °C. If the rise continues into the twenty-second century, the global average temperature may reach higher values than have occurred in the past 10 million years.

THE likelihood of global climate change from increasing concentrations of carbon dioxide is now well known¹⁻¹⁰. Much more recent is the realization that other less-abundant trace gases are also increasing and that their change is likely in total to have an effect on climate comparable with that of carbon dioxide¹¹⁻²¹. These include CH₄, tropospheric O₃, N₂O and the chlorofluorocarbons (CFCs) CCl₃F and CCl₂F₂. What do we know about the effect of these gases on climate, their temporal trends, and the implications of these increases? To see how such climate change can occur, we first consider the energy-exchange processes of the Earth-atmosphere system. The relative contribution of the various trace gases to climate change will be determined by their relative contributions to these energy processes.

Solar radiation is absorbed by the atmosphere and the Earth's surface, providing the energy required by many terrestrial processes. Figure 1 shows the amount of energy involved, on a global and annual average. For climate to be in equilibrium, this absorbed solar radiation must be balanced by outgoing thermal radiation. The partial trapping of this thermal radiation by radiatively-absorbing particles or molecules helps to increase surface temperatures by several tens of degrees compared with what they would be without the atmosphere. This process, sometimes called the 'greenhouse effect', occurs largely in the

first 10–15 km of the atmosphere, that is, the troposphere. Radiatively active constituents both absorb and emit thermal radiation, depending on their effective cross-sections and on temperature. Their emission varies with the local atmospheric temperature, whereas their absorption depends on the temperatures of the other atmospheric layers and of the Earth's surface where the radiation was initially emitted. Because tropospheric temperatures decrease with increasing altitude, typically by 5–7 °C km⁻¹, the active atmospheric constituents absorb more upward radiative flux than they emit. Clouds and water vapour are the dominant contributors to this process.

What is surprising is that other atmospheric gases, present in minute amounts, in some cases in concentrations <1 part per 10⁹ (p.p.b.), also contribute significantly to this trapping of thermal radiation. These gases include, in particular, carbon dioxide, tropospheric ozone, methane, nitrous oxide, and certain chlorocarbons, in order of their present contributions to atmospheric radiative processes. Without the atmosphere's radiatively active constituents, the Earth would lose thermal radiation essentially as a black body at the temperature of its surface, $T_s = 287.5$ K, that is, ~ 387 W m⁻², neglecting corrections for the

Table 1 The current 1985 trapping of thermal infrared radiation (ΔQ) by current tropospheric trace constituents

Gas	Current concentration	ΔQ_{total} (W m ⁻²)	Ref.
Carbon dioxide	345 p.p.m.	~ 50	63
Methane	1.7 p.p.m.	1.7	16
Ozone	10–100 p.p.b.	1.3	
Nitrous oxide	304 p.p.b.	1.3	16
CFC-11	0.22 p.p.b.	0.06	19
CFC-12	0.38 p.p.b.	0.12	19

The term ΔQ_{total} is the change of net radiation at the tropopause if the given constituent is removed, but the atmosphere is otherwise held fixed.

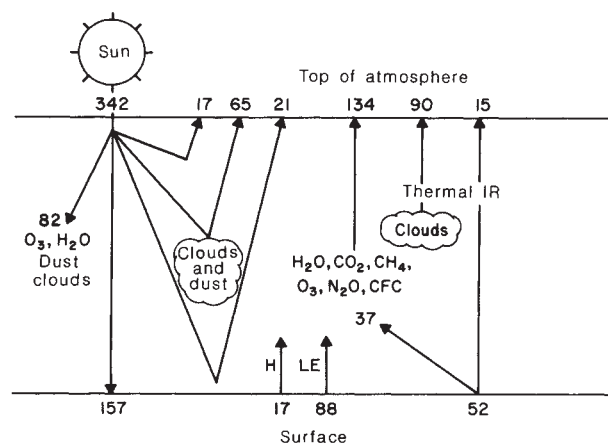


Fig. 1 Schematic depiction of the global and annual average fluxes of radiation within the climate system. Incident solar radiation is either absorbed or reflected as indicated on the left side. Most of the reflection occurs in the atmosphere and most of the absorption at the Earth's surface. Evapotranspiration (LE) and sensible heat fluxes (H) transfer much of the absorbed solar energy to the atmosphere. The absorbed solar energy must ultimately be returned to space as thermal infrared radiation as indicated on the right side. Only the net thermal flux between the surface and atmosphere is shown. Reductions of thermal flux to space from increasing concentrations of trace gases warm the global atmosphere.

nonlinearity of the black-body emission, surface emissivities <1, and stratospheric effects. The actual radiative flux from the top of the atmosphere is 239 W m⁻², so the total amount of trapped radiation is ~ 148 W m⁻². Sustained changes in this trapping by as little as 1 W m⁻² change the Earth's radiative balance sufficiently to be of considerable importance for the climate system. Table 1 shows the current concentrations of atmospheric trace gases and contributions to this radiative trapping.

The contribution to the Earth's thermal budget of such minute concentrations of trace gases is of considerable interest in its own right. However, the changing concentrations of these gases as a result of human activities^{20,21} add a considerable sense of urgency to their study. We review here our current understanding

of the radiative properties of the relevant gases, their sources, sinks, and changing concentrations, and the implications of these changes for future climate.

Radiative properties of the atmosphere

Solar radiation of wavelengths from ~ 0.3 to $\sim 4 \mu\text{m}$ heats the climate system. Thermal emission at wavelengths from ~ 4 to $\sim 100 \mu\text{m}$, in turn, cools the Earth's surface and atmosphere, ultimately returning the absorbed solar energy to space. The solar radiation is scattered (reflected in all directions) by clouds, aerosols, the Earth's surface, and the major atmospheric molecules (Fig. 1) and absorbed by all but the last of these. Thick clouds act nearly as black bodies in absorbing and emitting thermal infrared radiation, as do also most terrestrial surfaces (an exception is quartz sand with relatively low emissivities²²).

Of the radiatively active gases in the current atmosphere, only H_2O , CO_2 , CH_4 , O_3 , N_2O and the chlorofluorocarbons (CFC-11 and CFC-12) are in sufficient concentrations to be important in Earth's overall thermal budget. Of these, the strongest absorber by far is water vapour. Indeed, much of tropospheric radiation can be described with water vapour as the only gaseous absorber. Water in either vapour or in cloud form absorbs solar radiation and absorbs and emits thermal radiation. These water vapour states are internal to the climate system, that is, their distributions are controlled by climate processes themselves (that is, the atmospheric hydrological cycle) rather than by sources uncoupled to the climate system. Understanding how water vapour concentrations and cloud radiative properties might change in the future thus requires knowledge of how these terms function as feedbacks in the climate system (a question we shall return to later). Our primary thrust here is to review the role of the other less-abundant trace gases, whose concentrations are being directly perturbed by human activities. Understanding the radiative properties of these gases and their future concentrations is the key to understanding the relative contributions of these gases to climate change.

Ozone is the only other atmospheric gas which absorbs much solar radiation, primarily in the stratosphere. Atmospheric carbon dioxide is but a very weak absorber of solar radiation. The other atmospheric trace gases only affect the atmospheric heat budget through their absorption and emission of the thermal infrared radiation, primarily over the wavelength range ~ 6 – $16 \mu\text{m}$. Furthermore, at the wavelengths where water vapour strongly absorbs and emits radiation, the effect of other gases is minimal. The vibrational-rotational bands of water vapour thus block radiation at wavelengths $< 8 \mu\text{m}$ and rotational bands block wavelengths $> 18 \mu\text{m}$. Carbon dioxide, in turn, dominates the absorption of radiation between 12 and $18 \mu\text{m}$.

The remaining spectral region from 8 to $12 \mu\text{m}$ is known as the 'window' because of the atmosphere's relative transparency to radiation over these wavelengths. The intensity of black-body radiation depends on wavelength according to the Planck function; at the temperatures of the Earth's surface, this function has its maximum values in the window region. Consequently, $\sim 25\%$ of the thermal emission from the Earth's surface ($\sim 100 \text{ W m}^{-2}$) is at wavelengths of 8– $12 \mu\text{m}$. A larger fraction of the emission from the top of the atmosphere is in this spectral range. In this wavelength region, emission varies with temperature T approximately as $\exp(-1,500/T)$. A trace gas at a temperature $\sim 33^\circ\text{C}$ less than that of Earth's emission temperature will thus only re-emit about half as much energy as it absorbs. Because of the presence of clouds, the maximum increase of trapping in the window region is $\sim 30 \text{ W m}^{-2}$.

Interestingly enough, CH_4 , O_3 , N_2O , CFC-11 (CCl_3F) and CFC-12 (CCl_2F_2) all have strong absorption bands in the atmospheric window region. These trace gases absorb and emit as functions of wavelength in discrete lines with extended wings. These lines occur in bands and result from the rotational splitting of individual vibrational energy transitions of the molecules. Their strength is determined from the strength of the band

transitions, from the concentration of the trace gas, and from the rotational partitioning of the band into individual lines. The emission rate of a line depends on its absorption strength and on local atmospheric temperature.

The weakest lines absorb radiation significantly only in their line cores, and this absorption increases essentially linearly with concentration of the absorber gas. Somewhat stronger lines absorb radiation mostly in the wings, with only relatively small amounts absorbed by their saturated cores. Increasing absorber concentration pushes the peak absorption farther into the line wings so that wing absorption increases with the square root of the product of atmospheric pressure and absorber concentration. With even stronger line strengths, the peak absorption is pushed so far into line wings that other lines within the band overlap the absorbing wings and absorption only increases logarithmically with increasing absorber concentration. The absorption of a band varies with absorber concentration according to the variation of its stronger absorbing lines.

The chlorofluorocarbons CFC-11 and CFC-12 and tropospheric ozone are present in such small concentrations and have so many individual lines that their absorption of thermal radiation is nearly proportional to their concentration. Methane and nitrous oxide, being relatively more abundant, increase their absorption essentially according to the square root of their concentrations, whereas carbon dioxide absorption is proportional to the logarithm of its concentration. All the trace gases absorb not in single bands but in multiple bands, and weaker hot and isotopic bands are also present. The above scaling arguments are only approximate and are especially prone to error if applied to large changes in concentration where either the stronger bands may change their dependence on absorber amount, or previously unimportant weaker bands of a given gas may become relatively more important.

Increases in the concentrations of atmospheric trace gases since pre-industrial times and the consequent trapping of thermal radiation are shown in Table 2. The trapping by ozone and

Table 2 Estimated pre-industrial trace gas concentrations and implied change in thermal trapping to 1985

Gas	Pre-industrial concentration	$\Delta Q_{\text{pre-industrial}}$ (W m^{-2})
Carbon dioxide	275 p.p.m.	1.3
Methane	0.7 p.p.m.	0.6
Tropospheric ozone (below 9 km)	0–25% less	0.0–0.2
Nitrous oxide	285 p.p.b.	0.05
CFC-11	0.00 p.p.b.	0.06
CFC-12	0.00 p.p.b.	0.12
Total		~ 2.2

Trapping is inferred from refs 19 and 20, scaling approximately logarithmically, with CO_2 concentration, with the square root of N_2O and CH_4 concentration, and linearly with O_3 and CFC-11 and -12 concentrations.

the CFCs, whose stronger bands are in the middle of the window region, is affected very little ($\sim 15\%$) by overlap with water vapour and with carbon dioxide absorption. Methane and nitrous oxide, whose strongest bands are on the short wavelength edge of the window region, lose about half of their trapping²⁰ by such overlap. Since much of the effect of increases in the trace gases is to make the atmospheric window region more opaque, this trapping might be called the 'dirty window' effect.

Atmospheric trace gases

Carbon dioxide: Atmospheric carbon dioxide concentrations are now $\sim 25\%$ higher than they were 200 yr ago before the age of extensive industrialization and forest clearing. Current concentrations (1985) are about 345 p.p.m. (parts per 10^6) and are increasing by 1 – $1.5 \text{ p.p.m. yr}^{-1}$, mostly as a result of continued

burning of fossil fuel (adding $\sim 5.2 \times 10^{12}$ kg yr⁻¹ of carbon) and forest removal (continuing to add about 10^{12} kg yr⁻¹ of carbon)¹⁰. The basic features of the global carbon cycle are well known, but questions remain as to the contributions of land soils and forests and the rate of uptake of carbon by the oceans. The largest source of uncertainty in projecting future atmospheric carbon dioxide concentrations, however, lies in the future rates of fossil fuel combustion.

Halocarbons: Halogenated hydrocarbons (halocarbons) are now widely produced and widely used. These include fluorocarbons, chlorofluorocarbons, chlorocarbons and bromocarbons. Most scientific attention has focused on the potential role of chlorine and bromine atoms from these molecules as ozone-destroying catalysts in Earth's stratosphere²³⁻²⁵. Certain of these chemicals have also been recognized to be potent greenhouse gases^{11,20}.

The chlorofluorocarbons, often referred to popularly by their trade name of 'Freons', CCl₃F (CFC-11) and CCl₂F₂ (CFC-12), were measured first in the atmosphere in 1971 (ref. 26) and 1973 (see, for example, ref. 27), respectively. Since then, increasingly accurate and precise measurements have shown that their atmospheric concentrations have grown at about the same rate as would be expected from industrial production and release into the atmosphere^{24,28}, after minor corrections for the amounts produced but not yet released and for the quantities destroyed in the stratosphere and dissolved in oceans. In 1981, there were about 6.0×10^9 kg of CFC-12 in the atmosphere together with 5.0×10^9 kg of CFC-11. At 1981 release rates, these amounts represent the cumulative results of 15–20 yr of global emissions. (Unpublished data from several investigators give 1985 mixing ratios of CFC-11 of at least 0.22 p.p.b. and for CFC-12 of at least 0.38 p.p.b.). Indeed, atmospheric concentrations of these compounds were observed to have increased by $\sim 6\%$ yr⁻¹ during 1978–81 (refs 29, 30). In the early 1970s, annual emissions were a larger fraction of the amounts already in the atmosphere, and larger percentage annual increases were observed, along with considerably more CFCs in the Northern Hemisphere than in the Southern Hemisphere. Although most of the release continues to be in the Northern Hemisphere, the hemispheric difference has decreased to $<10\%$.

A projection of concentrations of atmospheric CFC-11 and CFC-12 beyond the present requires some knowledge about future industrial production and release rates. Current releases annually increase mixing ratios of CFC-11 by about 12 p.p.t. (parts per 10¹²) and CFC-12 by 21 p.p.t., as estimated from changing atmospheric concentrations. A recent report³¹ attempts to estimate their future production and release rates and that of several other similar chemicals; it recognizes the dynamics of changing industrial usages, competitive products and world and regional populations. Worldwide CFC-11 emissions are projected to increase by between 2.8 and 5.1% yr⁻¹ from 1980 until 2000; corresponding figures for CFC-12 are 2.5–3.5% yr⁻¹. Between AD 2000 and 2025, CFC-11 emissions could change by -1% to $+4.7\%$ yr⁻¹ and CFC-12 emissions would increase by between 1.0 and 4.6% yr⁻¹. Beyond the year 2025, growth estimates are less well-based, but Quinn *et al.*³¹ suggest annual increases of 0.6–2.0% for CFC-11 and 1.1–2.1% yr⁻¹ for CFC-12.

Even if future release rates of CFC-11 and CFC-12 remained at present levels, their atmospheric concentrations would continue to increase for the next century because of the relatively long residence times, τ , for these species, at least 60 and 100 yr, respectively³². In general, for a well-mixed chemical with sources $S(t)$

$$\frac{dN(t)}{dt} = S(t) - N(t)/\tau \quad (1)$$

where $N(t)$ is the total number of molecules (or mass) of the given species in the global atmosphere at time t . Even if we were to assume that present emissions remained constant, equation (1) shows that concentrations of CFC-11 and CFC-12

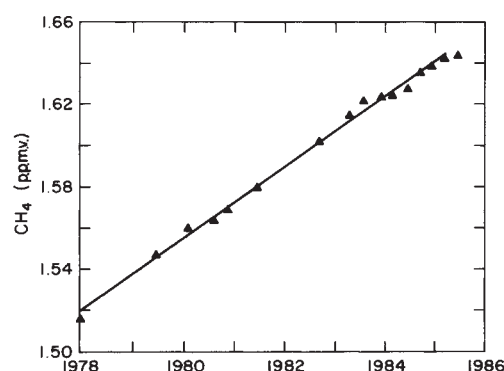


Fig. 2 Globally averaged methane concentrations in surface air from January 1978 to June 1985 as measured by Blake and Rowland⁴². Air samples were collected simultaneously from northern and southern latitudes. In computing global averages, measured values were weighted by the Earth's area in latitude belts. In 1985, there are 4.8×10^{15} g CH₄ in the atmosphere; annual sources (sinks) are $(3-7) \times 10^{14}$ g.

would more than double, to values of ~ 0.5 and 1.0 p.p.b. respectively, within the next 40 yr and eventually rise to values of ~ 0.7 and 2.1 p.p.b., using 60- and 100-yr lifetimes, respectively.

Other halocarbons requiring attention include CCl₄, CClF₃, CF₄, CHClF₂, CHCl₂F, C₂H₃Cl₃, C₂Cl₃F₃, C₂Cl₂F₄, C₂ClF₅, C₂F₆, CBrF₃ and CBrClF₂. Because of the location of the absorption bands of these species and their rapid rates of accumulation in the atmosphere, they represent potentially important greenhouse gases. Research is needed to quantify their band absorptances and atmospheric concentrations with special attention to CHCl₂F and C₂Cl₃F₃.

Nitrous oxide: Atmospheric N₂O is increasing in concentration globally, although the record of its past change is not as extensive as that for methane, and the reasons for this increase are less clear than those for halocarbons. Weiss³³ measured a global increase of ~ 0.6 p.p.b. yr⁻¹ (0.2% yr⁻¹) between 1976 and 1980, whereas Khalil and Rasmussen³⁴ find an 0.8 p.p.b. yr⁻¹ increase between 1979 and 1982. The mid-1980 N₂O concentration in the Northern Hemisphere was 301 p.p.b. and in the Southern Hemisphere it was ~ 0.8 p.p.b. less. Weiss fits his 1976–80 data with one of two curves; either one that assumes an added combustion source of N₂O growing at 3.5% yr⁻¹ or one that assumes an agricultural-fertilizer source growing at 6% yr⁻¹ (ref. 33) with an atmospheric residence time of 100 yr in both cases. Weiss, in extending his N₂O monitoring through 1984, finds that global concentrations continue to rise by 0.6 p.p.b. yr⁻¹ (personal communication), but that it is not yet possible to distinguish between the two source-growth curves that fit the data. Earlier data from 1961–74 showed similar rates of N₂O increase³⁵.

Although the present observed and projected rates of N₂O increase may seem small, they imply that total global sources of N₂O are now elevated by $\sim 30\%$ over unperturbed steady-state sources, assuming a growth rate of 0.2% yr⁻¹ and a 150-yr lifetime for N₂O. If the N₂O increase is due to either increased microbial nitrification and denitrification of agricultural fertilizers, or combustion, then much of this imbalance has developed in the past few decades and it may continue to grow rapidly.

Concentrations of N₂O of 320–330 p.p.b. are likely by AD 2000, regardless of which anthropogenic source is assumed to be growing^{33,34}. Better future projections require determination of whether increased combustion of nitrogen-containing fuels^{36,37} and biomass²¹ or increased application of nitrogen fertilizers³⁸ is primarily responsible for the continuing N₂O increase. Both sources are difficult to quantify; field losses of N₂O due to microbial nitrification and denitrification of nitrogen fertilizers are variable^{38,39} and candidate elementary reactions

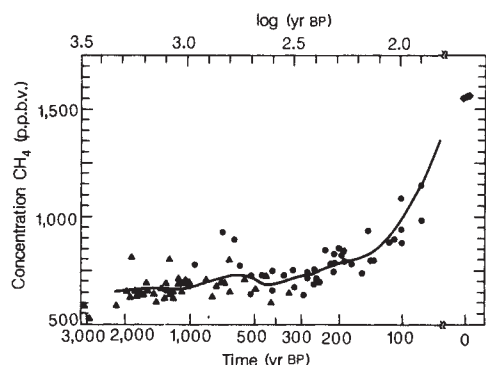


Fig. 3 Concentrations of atmospheric methane (100–300 yr BP) from ref. 44. ●, Data from polar ice cores obtained from Greenland; ▲, data from ice cores at the South Pole. The present concentration of methane is shown on the right (◆). The solid line is the smoothed average concentration based on the data; it is the average concentration in successive overlapping periods of 0.3 units each in the log (l) scale at the top of the figure.

that can produce N_2O in combustion have been identified only recently⁴⁰.

Methane: Methane is of considerable scientific interest because of its relatively rapid growth and the diverse possible causes of this increase. Global concentrations have increased by $>1\% \text{ yr}^{-1}$ since 1978, as shown by Fig. 2, based on data from flame-ionization gas chromatography. A reanalysis⁴¹ of ground-based solar absorption infrared spectroscopic data concludes that the concentration of tropospheric CH_4 in 1951 was 1.15 p.p.m., about 30% below that of 1985 (ref. 42). An earlier re-examination of infrared data from the 1950s and 1960s had concluded⁴³ that the rate of methane increase was slower.

The increase in global methane apparently began in previous centuries. Figure 3 shows an analysis of gases trapped in dated ice cores⁴⁴, indicating that methane concentrations have doubled since the eighteenth century, but that they were relatively constant for the previous 2,000–3,000 yr. Selective diffusion of CH_4 out of old ice cores may have biased the data of Fig. 3 (ref. 43), but H. B. Craig, who earlier found similar results⁴⁵, considers this unlikely (personal communication) based on his tests. Further confirmation has been reported⁴⁶. Methane-consuming microbes may have lived in the ice, but we consider this unlikely.

Why has atmospheric methane increased in the past, and what is its future? Answering these questions requires knowledge of both the sources and sinks of methane. Ehhalt⁴⁷ summarized five measurements of ^{14}C in samples from air liquefaction plants in the 1950s. These samples had a ^{14}C content of about 80% of that of standard wood, or equivalently 20% 'dead carbon' from fossil fuels. He suggested that this value of 20% should only be used as an upper limit since air liquefaction plants are usually situated in heavily industrialized areas so that the samples were subject to contamination from local fossil-fuel-derived methane. Various authors over the past decade have constructed methane-source inventories subject to this constraint. A recent examination²¹ lists rice paddies, ruminant animals, biomass burning, swamps and marshes, natural gas, and coal mining losses as the major sources, in decreasing order of importance. However, serious questions of mechanisms and related ecological variability remain^{48–51}. Further, we should be cautious about conclusions drawn from the 1950s ^{14}C data because: first, the relative sizes of ^{14}C -rich and ^{14}C -poor sources are probably changing with time; and second, the old samples, collected by liquefying air, may not be representative of the $^{14}\text{CH}_4/^{12}\text{CH}_4$ in air. Unfortunately, few conclusions can be drawn about methane sources from ^{14}C analyses today in view of the bomb-produced ^{14}C in the contemporary biota and sediments. It might be useful to examine the concentrations of the ^{13}C stable isotope; such research is just beginning⁵².

The principal known sink of atmospheric methane is the

gas-phase reaction $\text{OH} + \text{CH}_4 \rightarrow \text{CH}_3 + \text{H}_2\text{O}$ (ref. 72). Some tantalizing but indirect evidence suggests that atmospheric OH concentrations are decreasing⁵³, at least in NO poor regions—the lower and middle troposphere; increasing methane and increasing CO concentrations (not yet proven) should act to suppress OH (ref. 54) through nonlinear atmospheric chemistry.

Ozone: Ozone is of considerable interest because of its important roles, its complex behaviour, and its susceptibility to human-induced global change. Near the ground in urban areas, ozone is a pollutant. Throughout the global troposphere, ozone photolysis initiates free-radical chemistry. In the middle and upper stratosphere, where the largest concentrations are found, ozone strongly attenuates solar ultraviolet light and in doing so drives stratospheric wind systems.

Tropospheric ozone increases are difficult to discern with extant observing systems, but increases have been indicated. A recent critical analysis⁵⁵ of ozone data finds that, on average over Europe and North America from the late 1960s to the early 1980s, near-surface ozone has increased by $1\text{--}3\% \text{ yr}^{-1}$ and ozone in the middle troposphere (700–500 mbar) by $1\text{--}2\% \text{ yr}^{-1}$. These increases occur largely during the summer, especially in industrialized areas. Such increases might have begun in the 1940s, as is suggested by shifts in the seasonal cycle of ozone concentrations and other scattered observations⁵⁵. There is considerable variability and little evidence for change in the upper troposphere.

Theoretical models of tropospheric chemistry predict that increasing anthropogenic releases of hydrocarbon and nitrogen oxide (NO_x) gases should be increasing tropospheric ozone^{55–57,15}. Such increases depend on poorly known atmospheric NO_x concentrations and on some meteorological processes such as vertical transport and chemical roles of clouds, but changes in the Northern Hemisphere should be larger than those in the Southern Hemisphere, as is evidently observed. Future changes in the stratosphere may also lead to increases of tropospheric ozone.

As discussed previously²⁰, most studies of the latitudinal variation of tropospheric ozone indicate that concentrations in the Northern Hemisphere are at least 50% higher than in the Southern Hemisphere. However, a recent aircraft survey has given contradictory results⁵⁸, possibly because of seasonal or longitudinal variations. Furthermore, latitudinal variations may be due to transport⁵⁹.

An association of observed trends of ozone in the Northern Hemisphere with increases in total fossil fuel use would also support the possibility of an increase of tropospheric ozone in the Northern Hemisphere of up to 50% over pre-industrial values. We indicate in Table 2 that global average tropospheric ozone (below 9 km) is estimated to have increased by between 0 and 25% since pre-industrial time. Improving estimates of past or future ozone change will require a better understanding of the contributions of ground and high-level pollution.

Global stratospheric ozone changes are likely to occur because of continuing and accelerating human activities^{24,25}. Increasing concentrations of chlorofluorocarbons and nitrous oxide decrease stratospheric O_3 at altitudes above 30 km. Changes of ozone in the lower stratosphere are more complex and difficult to project; they will depend on latitude and altitude and on wind systems^{59,60}. A variety of chemical reactions involving polyatomic species (many of which have yet to be measured in the atmosphere) also affect this response.

Future scenarios

For comparing the future climate effects of the various trace gases, we take the year 2050 as a reference point and assume the ranges indicated in Table 3. The question of possible future concentrations for CO_2 has been extensively studied, and we have adopted values from the latest such study¹⁰.

A range of possible future emissions for CFC-11 and CFC-12 has been obtained from economic analysis³¹, and we have used

these emissions together with equation (1) to obtain the concentrations shown in Fig. 4. Concentration scenarios have taken $\tau = 60$ yr for CFC-11 and $\tau = 100$ yr for CFC-12, near the low end of most previously inferred values³². Lifetimes are likely to lie in the range 50–80 yr and 90–130 yr, respectively. These lifetimes will decrease in the future, as increased concentrations of chlorine atoms lower the ozone concentrations above 30 km, and hence increase the fluxes of ultraviolet that photolyse the CFCs. Obvious decreases in the total column ozone following stratospheric ozone decrease are likely to trigger political constraints on further increases in the production of the CFCs. Our projections neglect the uncertainty in CFC lifetimes and their decrease with increasing burden and also neglect possible emission reductions from future regulation of CFC production.

Table 2 shows that the radiative effect of the CFCs which have been added to the atmosphere up to 1985 is only ~11% of that from CO₂ increases. However, the CO₂ increase has occurred over the past two centuries, and the current burden of CFCs has been added almost entirely within the past 30 yr. The radiative effect of these CFCs added over the past 30 yr is about one-third that of the CO₂ added over the same period. Table 3 indicates that for the assumed future scenario the additional radiative trapping by the CFCs between 1985 and 2050 is likely to be ~70% that of CO₂. This increase in the relative importance of the CFCs in the future follows from the near-linear dependence on concentration of the CFC radiative effects versus the logarithmic dependence of the effects of CO₂.

Because of our current relatively poor understanding of the reasons for the increases in methane concentrations, a wide range of possible futures appears in Table 3. A continuation of the current growth rate of 1.2% yr⁻¹ to the year 2050, assuming present methane lifetimes, would give 3.7 p.p.m. at that time. Increases in methane will probably be amplified by decreasing OH, but will be diminished by slowdown in population growth and required resources. Another recent analysis²¹ argues that increases from pre-industrial concentrations are likely to be proportional to projected future populations. They accordingly infer concentrations of 2.5 p.p.m. by 2050; values this small would require a large reduction in methane growth rates within the next few decades. We judge that in the year 2050 concentrations will lie between 2.1 and 4.0 p.p.m. The upper limit is inferred from the extrapolation of current growth plus a small decrease (8%) of methane lifetimes. Methane lifetimes may decrease by much more⁵⁴. The lower limit is inferred by arguing that the current trend should last at least 20 yr but that, considering our ignorance of the processes involved, we cannot exclude absence of further growth after that. Table 3 suggests that from now to 2050 the radiative effects of increasing methane are likely to be about one-quarter that of CO₂. Table 2 indicates that the increase in radiative trapping from past methane increases has been about the same as that expected from future methane increases and is about half of that inferred from past CO₂ increases. Methane has already increased to ~250% of pre-industrial values and probably will not repeat this relative increase by 2050. The relative increase of CO₂, on the other hand, will probably be greater in the next 65 yr than it has been up to now¹⁰.

If the anthropogenic source of nitrous oxide were to continue to grow at 3.5% yr⁻¹, it would increase by a factor of 10 by the year 2050 and N₂O concentrations would rise above 500 p.p.b. (ref. 33). Such rapid future growth is probably excluded by economic and population constraints²¹. Assuming that a quadrupling of the current emission rates over the next 65 yr is not impossible, we infer an upper limit for N₂O concentrations of 450 p.p.b. for the year 2050. We infer a lower limit of 350 p.p.b. for the year 2050 using an average annual increase similar to that now observed.

A likely upper limit to fossil-fuel consumption over the next century is about four times current consumption rates¹⁰. With such consumption and an upper limit of 20% of current ozone

associated with fossil-fuel pollution, global tropospheric ozone could conceivably increase by 60% over current values. Ozone will also increase in the upper troposphere and lower stratosphere in response to increases of the CFCs and to oxides of nitrogen from increasing jet aircraft traffic. The details of latitudinal and especially vertical distribution of ozone change are important for any accurate estimate of climate effects. Lacking such details, we estimate the likely radiative trapping of changing ozone concentrations by the year 2050 as that given by a change in ozone between 0 and 50% uniformly over the altitude range 0–12 km.

To determine the net contribution to radiative trapping included in Table 3, we have simply summed separately the upper and lower limits of the projected contributions of the individual gases. Although all contributions are unlikely to achieve their upper or lower limits simultaneously, this assumption probably does not overestimate the likely range of total trapping for two reasons. First, the rates of increase of the different trace gases are more likely to be highly correlated than not—their sources all depend heavily on fossil-fuel utilization; methane and nitrous oxide emissions both also depend on agricultural development and that of the CFCs on general technological development. Second, past experience with similar

Table 3 Scenario for trace gas concentrations in the year 2050 and implied increased trapping of thermal radiation from 1985 (ref. 19)

Gas	Year 2050 scenario	ΔQ_{2050} (W m ⁻²)
Carbon dioxide	400–600 p.p.m.	0.9–3.2
Methane	2.1–4.0 p.p.m.	0.2–0.9
Tropospheric ozone (0–12 km)	15–50% more	0.2–0.6
Nitrous oxide	350–450 p.p.b.	0.1–0.3
CFC-11	0.7–3.0 p.p.b.	0.23–0.7
CFC-12	2.0–4.8 p.p.b.	0.6–1.4
Total		2.2–7.2

uncertain environmental questions indicates that actual uncertainties are likely to be larger than can be judged from simple arguments such as those used here. In particular, there are many other trace gases present in small but rapidly growing concentrations²⁰, some of which will add significantly to global warming by the year 2050.

Referring to either the lower or the upper limit totals in Table 3, we infer that the total contribution of the other trace gases is more likely than not to exceed the contribution from CO₂.

Implications for climate change

We have aimed to provide an overall framework for considering the relative contributions of various trace gases to climate change. Relatively realistic three-dimensional climate models have been used to study only a very limited number of scenarios for future climate; that is, scenarios assuming a steady-state doubling or quadrupling of atmospheric carbon dioxide concentrations. The radiative forcing for the latter is about double that for the former.

The future climate change from likely increases of all trace gases will undoubtedly differ in detail from that inferred from the superposition of the climate changes that would occur, assuming that each trace gas changes separately. Furthermore, the climate change from individual trace gases depends not only on the global average trapping of radiation as discussed here, but also on the latitudinal and especially vertical distributions of atmospheric heating or cooling changes implied by the trace gas change. However, the implications of these details have not yet been satisfactorily resolved, and so our estimates of the climate change resulting from the various trace gases assume that the dependence of this climate change on external forcing can simply be represented by the global radiative trapping alone.

Indeed, the radiative trapping values given in Tables 1–3 are only approximate estimates. Their precise values depend not only on (somewhat uncertain) band strengths but also on the temporally and spatially varying distributions of cloudiness and atmospheric temperatures. The values used here have been inferred mostly from simple one-dimensional models of the atmosphere. Recognizing these caveats, we suggest that at present the most useful way to estimate the future climate change from CO₂ and the other trace gases is to scale from the studies that have been done on climate change from CO₂ increases, allowing for the time lag required for the oceans to warm up.

Three-dimensional general circulation models (GCMs) represent the climate system as the sum of the day-to-day weather systems integrated over many years of simulated time. In doing so, they can include realistically all the feedbacks of simpler models plus those that depend on the details they generate. They include, in particular, albedo feedbacks from seasonally varying snow and sea ice, lapse rate feedbacks involving adjustment to moist adiabatic lapse rates in tropical latitudes, controls by baroclinic disturbances in mid-latitudes, and feedbacks by the highly stable stratification of polar latitudes. Cloud feedbacks are potentially very important for amplifying global temperature change, but they cannot be inferred yet with confidence, and negative feedbacks on solar radiation absorption from changes in cloud liquid water have not been included⁶¹.

Because of the difficulty in isolating individual feedbacks in GCMs, and the expense of examining all trace gases of possible significance, simpler energy balance⁶² and convective adjustment models⁶³ have also proved valuable.

Recent GCM simulations^{3,6,7} give for steady-state CO₂ doubling ($\Delta Q \approx 4.0 \text{ W m}^{-2}$) a global average temperature increase of between 2.1 °C (ref. 3) and 4.8 °C (ref. 6). Consideration of uncertain model feedbacks suggests that a value as low as 1.5 °C or as high as 5.5 °C is possible¹⁰.

Because several decades or more are required for the oceans to equilibrate with changes in global radiation^{64–69}, we cannot convert the heating rates given in Tables 2 and 3 directly into expected temperature changes for the same dates. However, continuing the current burden of trace gases added by human activities indefinitely implies an eventual increase from pre-industrial temperatures of 0.5–2 °C. Global mean temperatures can only be estimated with any confidence back to 1900. About two-thirds of the anthropogenic forcing has developed since that time. Oceanic thermal inertia is currently reducing the temperature response by (very roughly) half of what it would be in a steady state. Allowing for this factor, we infer a temperature increase of 0.3–1.0 °C from 1900 to the present^{10,70}. The observed increase is about 0.5 °C (refs 10, 70) which would seem to confirm the present analysis. However, global temperatures around 1940 were nearly as warm as now and they declined in the 1960s. Thus, unexplained climate variations occur that will probably confound unambiguous identification of the trace gas signal for at least another decade or two.

The scenarios given in Table 3 imply that by the year 2050 there will be a global average temperature increase of 1 °C to >5 °C, depending on the extent of positive feedbacks in the climate system and on reduction of the response by oceanic heat uptake. Global temperatures will probably continue to grow well into the twenty-second century as the atmospheric loading of trace gases and ocean heat content increases further.

The GCMs simulate not only global conditions but also the detailed geographical distribution of many climate parameters including the atmospheric hydrological cycle. Implications of future climate change from trace gases for agriculture and natural ecosystems require establishing these details with some confidence. All the GCM simulations indicate largest temperature changes in high latitudes. Some models strongly suggest a significant mid-latitude, mid-continent summer drying. Past model studies have inspired a wide range of climate-impact 'what if' studies, examining the potential implications of the

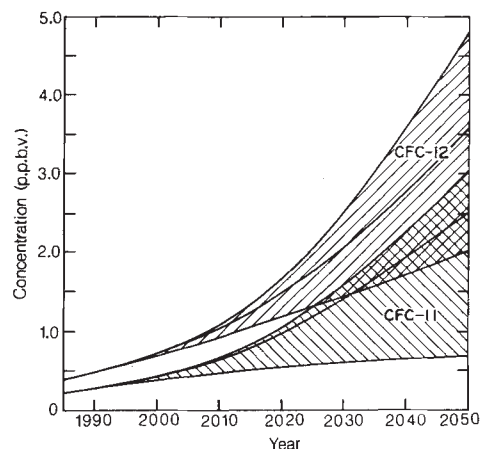


Fig. 4 Range of possible scenarios of the concentrations of CFC-11 and CFC-12 between 1985 and 2050 as inferred from future sources projected from an economic analysis³¹. Upper, lower and middle scenarios are shown for each gas and their ranges are indicated by the slanted line patterns.

range of possible future climates⁷³. However, practical analyses will require a new generation of climate models whose global feedbacks and regional simulations can be accepted with confidence. They will also require allowance for the large-scale adjustments of human systems that can occur on the timescales involved⁷¹.

Accurate model predictions may take more than a decade to arrive. Meanwhile, further emphasis should be given to defining and bounding the uncertainties in future climate change⁷¹. Without a better understanding of the extreme upper limits to possible future climate change, we must view with considerable concern this global experiment now under way.

We thank Drs J. Kiehl, W. Clark, and P. Crutzen for helpful comments. The National Center for Atmospheric Research is sponsored by the NSF.

1. Callendar, G. S. *Q. Jl R. met. Soc.* **64**, 223–240 (1938).
2. Manabe, S. & Wetherald, R. T. *J. atmos. Sci.* **24**, 241–259 (1967).
3. Manabe, S. & Stouffer, R. J. *J. geophys. Res.* **85**, 5529–5554 (1980).
4. Climate Research Board *Carbon Dioxide and Climate: Scientific Assessment* (National Academy of Sciences, Washington DC, 1979).
5. Dickinson, R. E. *Carbon Dioxide Review 1982* (ed. Clark, W. C.) 101–133 (Oxford University Press, 1982).
6. Hansen, J. *et al. Maurice Ewing Symp. 5* (eds Hansen, J. & Takahashi, T.) (American Geophysical Union, Washington DC, 1984).
7. Washington, W. M. & Meehl, G. A. *J. geophys. Res.* **89**, 9475–9503 (1984).
8. Carbon Dioxide Assessment Committee *Changing Climate* (National Academy of Sciences, Washington DC, 1983).
9. Mitchell, J. F. B. *Q. Jl R. met. Soc.* **109**, 112–152 (1984).
10. Bolin, B., Döös, B. R., Jäger, J. & Warrick, R. A. (eds) *The Greenhouse Effect, Climatic Change and Ecosystems. A Synthesis of the Present Knowledge* (Wiley, Chichester, in the press).
11. Ramanathan, V. *Science* **190**, 50–52 (1975).
12. Wang, W. C., Yung, Y. L., Lasis, A. A., Mo, T. & Hansen, J. E. *Science* **194**, 685–690 (1976).
13. Dickinson, R. E., Liu, S. C. & Donahue, T. M. *J. atmos. Sci.* **35**, 2142–2152 (1978).
14. Flohn, H. *Carbon Dioxide, Climate and Society* (ed. Williams, J.) 227–238 (Pergamon, London, 1978).
15. Fishman, J., Ramanathan, V., Crutzen, P. J. & Liu, S. C. *Nature* **282**, 818–820 (1979).
16. Donner, L. & Ramanathan, V. *J. atmos. Sci.* **37**, 119–124 (1980).
17. Hameed, S., Cess, R. D. & Hogan, J. *J. geophys. Res.* **85**, 7537–7545 (1980).
18. Lasis, A., Hansen, J., Lee, P., Mitchell, P. & Lebedeff, S. *Geophys. Res. Lett.* **8**, 1035–1038 (1981).
19. World Meteorological Organization *WMO Global Ozone Research and Monitoring Project, Rep. No. 14 of the Meeting of Experts on Potential Climatic Effects of Ozone and Other Minor Trace Gases* (World Meteorological Organization, Geneva, 1982).
20. Ramanathan, V., Cicerone, R. J., Singh, H. B. & Kiehl, J. T. *J. geophys. Res.* **90**, D3, 5547–5566 (1985).
21. Bolle, H. J., Seiler, W. & Bolin, B. *The Greenhouse Effect, Climatic Change, and Ecosystems. A Synthesis of the Present Knowledge* Ch. 4 (Wiley, Chichester, in the press).
22. Prabhakara, C. & Dalu, G. *J. geophys. Res.* **81**, 3719–3724 (1976).
23. Molina, M. J. & Rowland, F. S. *Nature* **249**, 810–812 (1974).
24. National Academy of Sciences/National Research Council *Stratospheric Ozone Depletion by Halocarbons: Chemistry and Transport* (Washington DC, 1979).
25. Prather, M. J., McElroy, M. B. & Wofsy, S. C. *Nature* **312**, 227–231 (1984).
26. Lovelock, J. E., Maggs, R. J. & Wade, R. J. *Nature* **241**, 194–196 (1973).
27. Hester, N. E., Stephens, E. R. & Taylor, O. C. *J. Air Pollut. Control Ass.* **24**, 491–595 (1974).
28. Penkett, S. A., Brice, K. A., Derwent, R. G. & Eggleston, A. E. *J. Atmos. Envir.* **13**, 1011–1019 (1979).
29. Cunnold, D. M. *et al. J. geophys. Res.* **88**, C13, 8379–8400 (1983).
30. Cunnold, D. M. *et al. J. geophys. Res.* **88**, C13, 8401–8414 (1983).
31. Quinn, T. H. *et al. Projected Use, Emissions and Banks of Potential Ozone-Depleting Substances* WD-2483-1-EPA (Rand Corp., Santa Monica, 1985).

32. Stordal, F., Isaksen, I. S. A. & Horntvedt, K. *J. geophys. Res.* **90**, D3, 5757-5776 (1985).
33. Weiss, R. F. *J. geophys. Res.* **86**, C8, 7185-7195 (1981).
34. Khalil, M. A. & Rasmussen, R. A. *Tellus* **35B**, 161-169 (1983).
35. Craig, H., Weiss, R. F. & Dowd, W. L. *Pap. at Symp. on the Terrestrial Nitrogen Cycle and Possible Atmospheric Effects* (American Geophysical Union, Washington DC, 1976).
36. Pierrotti, D. & Rasmussen, R. A. *Geophys. Res. Lett.* **3**, 265-268 (1976).
37. Weiss, R. F. & Craig, H. *Geophys. Res. Lett.* **3**, 751-754 (1976).
38. Delwiche, C. C. (ed.) *Denitrification, Nitrification, and Atmospheric Nitrous Oxide* (Wiley, New York, 1981).
39. Mosier, A. R., Parton, W. J. & Hutchinson, G. L. *Environmental Biogeochemistry* (ed. Hallberg, R.) (1982).
40. Perry, R. A. *J. chem. Phys.* **82**, 5485-5488 (1985).
41. Rinsland, C. P., Levine, J. S. & Miles, T. *Nature* **318**, 245-249 (1985).
42. Blake, D. R. & Rowland, F. S. *J. atmos. Chem.* (in the press).
43. Ehhalt, D. H., Zander, R. J. & Lamontagne, R. A. *J. geophys. Res.* **88**, C13, 8442-8446 (1983).
44. Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **89**, D7, 11599-11605 (1984).
45. Craig, H. & Chou, C. C. *Geophys. Res. Lett.* **9**, 1221-1224 (1982).
46. Stauffer, B., Fischer, G., Neftel, A. & Oeschger, H. *Science* **229**, 1386-1387 (1985).
47. Ehhalt, D. H. *Tellus* **26**, 58-70 (1974).
48. Rasmussen, R. A. & Khalil, M. A. K. *J. geophys. Res.* **86**, C10, 9826-9832 (1981).
49. Cicerone, R. J., Shetter, J. D. & Delwiche, C. C. *J. geophys. Res.* **88**, C15, 11022-11024 (1983).
50. Seiler, W., Holzappel-Pschorn, A., Conrad, R. & Scharfe, D. *J. atmos. Chem.* **1**, 241-268 (1984).
51. Harriss, R. C., Gorham, E., Sebach, D. I., Bartlett, K. B. & Flebbe, P. A. *Nature* **315**, 652-654 (1985).
52. Stevens, C. M. & Rust, F. E. *J. geophys. Res.* **87**, C7, 4879-4882 (1982).
53. Khalil, M. A. K. & Rasmussen, R. A. *Atmos. Envir.* **19**, 397-407 (1985).
54. Thompson, A. M. & Cicerone, R. J. *Nature* (submitted).
55. Logan, J. A. *J. geophys. Res.* **90**, 10463, 10482 (1985).
56. Liu, S. C., Kley, D., McFarland, M., Mahlman, J. D. & Levy, H. II *J. geophys. Res.* **85**, 7546-7552 (1980).
57. Crutzen, P. J. & Gidel, L. T. *J. geophys. Res.* **88**, 6641-6661 (1983).
58. Gregory, G. L., Beck, S. M. & Williams, J. A. *J. geophys. Res.* **89**, 9642-9648 (1984).
59. Levy, H., Mahlman, J. D., Moxim, W. J. & Liu, S. C. *J. geophys. Res.* **90**, 3753-3772 (1985).
60. Isaksen, I. S. A. & Stordal, F. *J. geophys. Res.* **90** (in the press).
61. Somerville, R. C. J. & Rener, L. A. *J. geophys. Res.* **89**, 9668-9672 (1984).
62. North, G. R., Cahalan, R. G. & Coakley, J. A. *Rev. Geophys. Space Phys.* **19**, 91-122 (1981).
63. Ramanathan, V. & Coakley, J. A. *Rev. Geophys. Space Phys.* **16**, 465-489 (1978).
64. Bretherton, F. P. *Prog. Oceanogr.* **11**, 93-129 (1982).
65. Spelman, M. J. & Manabe, S. *J. J. geophys. Res.* **89**, 571-586 (1984).
66. Bryan, K., Komro, F. G., Manabe, S. & Spelman, M. J. *Science* **215**, 56-68 (1982).
67. Thompson, S. L. & Schneider, S. H. *Science* **217**, 1031-1033 (1982).
68. Schneider, S. H. & Thompson, S. L. *J. geophys. Res.* **6**, 3135-3147 (1981).
69. Wigley, T. M. L. & Schlesinger, M. E. *Nature* **315**, 646-652 (1985).
70. Wigley, T. M. L. *Climate Monitor* **13**, 133-148 (1985).
71. Clark, W. C. *Ecologically Sustainable Development of the Biosphere* (Publ. No. 26, IIASA, 1985).
72. Crutzen, P. J. *The Geophysics of Amazonia* (ed. Dickinson, R. E.) Ch. 8 (Wiley, New York, in the press).
73. Parry, M. L. (ed.) *Climatic Change* (Spec. Issue) **7** (1985).

ARTICLES

Masses of the satellites of Uranus

S. F. Dermott & P. D. Nicholson

Center for Radiophysics and Space Research, Space Sciences Building, Cornell University, Ithaca, New York 14853, USA

The dynamical theory used to obtain the masses of the uranian satellites from their orbital precession rates contains a fundamental error. The masses can be derived from the precession rates, but a correct analysis of the available data must allow for the fact that the eccentricities, inclinations and precession rates all vary considerably with time, due to mutual secular perturbations.

THE Voyager 2 spacecraft should encounter Uranus on 24 January 1986 and we will then obtain the first detailed images of this puzzling and poorly understood system. Visual observations by Sir William Herschel in 1781 and Lassell in 1851 revealed the presence of four major satellites of similar size: Ariel, Umbriel, Titania and Oberon¹. The comparatively small and innermost satellite, Miranda, was discovered photographically by Kuiper in 1948 (ref. 1). All of these satellites orbit the planet in direct, near-equatorial, near-circular orbits, but the dynamics of the system appear to be quite different from those of the satellite systems of Jupiter and Saturn. This difference has been insufficiently recognized, which has led to serious misinterpretations of the available observational information.

The radii and the masses of the satellites are presented in Table 1. Brown *et al.*² and Brown and Clark³ determined the satellite radii from photometric and radiometric measurements

using a version of the standard radiometric model developed for asteroids. The latest and most complete derivation of the satellites' orbital elements (Table 2) is due to Veillet⁴, who has also determined the masses of the four larger satellites from the observed pericentre precession rates^{5,6}. The striking feature of these results is the marked dichotomy in the derived satellite densities; the densities of Ariel and Umbriel are similar to those of the inner satellites of Saturn, and are consistent with a composition of 40% rock and 60% water-ice, but Titania and Oberon appear to have much higher densities, suggesting that the water-ice constitutes little more than a thin outer shell. Near-infrared reflectance spectra show that all five satellites have surfaces of water-ice contaminated with a dark spectrally neutral material showing spectral characteristics similar to those of, for example, carbonaceous chondritic material⁷.

A second unusual feature of the Uranus system is the fact that all five satellites have comparable non-zero orbital eccentricities. Since the prediction⁸ and discovery⁹ of active volcanism on Jupiter's satellite Io, it has been realized that tidal heating due to eccentricity damping may dominate the internal heat budgets of some satellites, so that the thermal evolution of some satellites cannot be understood without a concomitant understanding of their orbital evolution. This is certainly true for Io and may also be for Europa¹⁰⁻¹² and for the saturnian satellite Enceladus¹³. The observed orbital eccentricities and the calculated tidal damping timescales of the uranian satellites are shown in Table 2. It is puzzling that the inner satellites have tidal damping timescales much shorter than those of the outer satellites, probably much shorter than the age of the Solar System, even though all the orbital eccentricities are both finite and of similar magnitude^{14,15}.

We argue here that the problem posed by the satellite density distribution arises from a misunderstanding of the orbital

Table 1 Masses of the uranian satellites

<i>j</i>	Satellite	Radius* (km)	<i>m</i> / <i>M</i> [†] ×10 ⁵	<i>m</i> / <i>M</i> [‡] ×10 ⁵	Densities§ (g cm ⁻³)
1	Miranda	250 ± 110	~0.1	0.2 ± 0.2	—
2	Ariel	665 ± 65	1.8 ± 0.6	1.8 ± 0.4	1.3 ± 0.5
3	Umbriel	555 ± 50	1.1 ± 0.3	1.2 ± 0.5	1.4 ± 0.6
4	Titania	800 ± 60	3.2 ± 0.8	6.8 ± 0.8	2.7 ± 0.6
5	Oberon	815 ± 70	3.4 ± 1.0	6.9 ± 0.8	2.6 ± 0.6

* From ref. 2.

† Derived from the radii of Brown *et al.*² using a nominal density of 1.3 g cm⁻³. The quoted uncertainties in the masses take no account of the uncertainties in the densities.

‡ From ref. 4.

§ Derived from the radii of Brown *et al.*² and the masses of Veillet.

dynamics and may be imaginary. We also show that the observed eccentricities of the inner satellites, Miranda and Ariel, may be partly forced by the outer satellites and that these forced eccentricities may be maintained despite strong tidal damping.

Secular perturbations

In the jovian and saturnian systems, the orbital elements of many of the satellites (Io, Europa and Ganymede; Mimas, Enceladus, Tethys, Dione and Hyperion) are affected by orbit-orbit resonances. With the exception of a near-commensurability involving Miranda, Ariel and Umbriel, the Uranus system exhibits no true resonances of this type. In the absence of such resonant interactions, the long-term variations in the orbital eccentricity, e , and the inclination, I , of a satellite are largely determined by the secular terms in the satellite's disturbing function, that is, by those terms in the disturbing function that do not depend on the mean longitudes of any of the satellites. If we number the satellites from 1 to n in order of increasing semimajor axis, a , then the secular part of the truncated disturbing function, R_j , for the j th satellite can be written¹⁶

$$R_j = n_j a_j^2 \left[\frac{1}{2} A_{jj} e_j^2 + \sum_{\substack{k=1 \\ k \neq j}}^n A_{jk} e_j e_k \cos(\tilde{\omega}_j - \tilde{\omega}_k) + \frac{1}{2} B_{jj} I_j^2 + \sum_{\substack{k=1 \\ k \neq j}}^n B_{jk} I_j I_k \cos(\Omega_j - \Omega_k) \right] \quad (1)$$

where

$$A_{jj} = n_j \left[\frac{3}{2} J_2 \left(\frac{R_p}{a_j} \right)^2 - \frac{9}{8} J_2^2 \left(\frac{R_p}{a_j} \right)^4 - \frac{15}{4} J_4 \left(\frac{R_p}{a_j} \right)^4 + \frac{1}{4} \sum_{\substack{k=1 \\ k \neq j}}^n \frac{m_k}{M} \alpha_{jk} \tilde{\alpha}_{jk} b_{3/2}^{(1)}(\alpha_{jk}) \right] \quad (2)$$

$$B_{jj} = -n_j \left[\frac{3}{2} J_2 \left(\frac{R_p}{a_j} \right)^2 - \frac{27}{8} J_2^2 \left(\frac{R_p}{a_j} \right)^4 - \frac{15}{4} J_4 \left(\frac{R_p}{a_j} \right)^4 + \frac{1}{4} \sum_{\substack{k=1 \\ k \neq j}}^n \frac{m_k}{M} \alpha_{jk} \tilde{\alpha}_{jk} b_{3/2}^{(1)}(\alpha_{jk}) \right] \quad (3)$$

$$A_{jk} = -\frac{1}{4} \frac{m_k}{M} n_j \alpha_{jk} \tilde{\alpha}_{jk} b_{3/2}^{(2)}(\alpha_{jk}) \quad (4)$$

and

$$B_{jk} = \frac{1}{4} \frac{m_k}{M} n_j \alpha_{jk} \tilde{\alpha}_{jk} b_{3/2}^{(1)}(\alpha_{jk}) \quad (5)$$

In these expressions

$$\alpha_{jk} = \begin{cases} a_k/a_j & \text{if } k < j \\ a_j/a_k & \text{if } k > j \end{cases} \quad (6)$$

$$\tilde{\alpha}_{jk} = \begin{cases} 1 & \text{if } k < j \\ \alpha_{jk} & \text{if } k > j \end{cases} \quad (7)$$

n_j is the mean motion, $\tilde{\omega}_j$ is the longitude of pericentre, Ω_j is the longitude of the ascending node, and m_j is the mass, all for the j th satellite. M , R , J_2 and J_4 , are, respectively, the mass, the radius and the first two zonal gravity coefficients of the planet, and the $b_s^{(k)}$ are Laplace coefficients¹⁶. Since J_2 is non-zero, the orbital inclinations of the satellites must be referred to the equatorial plane of the planet. In writing these expressions, we have neglected solar perturbations (negligible for the uranian satellites), terms of fourth order and higher in the satellite eccentricities and inclinations, and terms of second order in the masses.

The solution of Lagrange's equations for the orbital element variations produced by this disturbing function follows the

method described by Brouwer and Clemence¹⁶. It is convenient to transform the conventional orbital elements into the symmetrical forms

$$\begin{aligned} h &= e \sin \tilde{\omega} \\ k &= e \cos \tilde{\omega} \end{aligned} \quad (8)$$

and

$$\begin{aligned} p &= \sin I \sin \Omega \\ q &= \sin I \cos \Omega \end{aligned} \quad (9)$$

in terms of which the solutions take the simple harmonic form:

$$h_j = \sum_{i=1}^n e_{ji} \sin(g_i t + \beta_i) \quad (10)$$

and

$$p_j = \sum_{i=1}^n I_{ji} \sin(f_i t + \gamma_i) \quad (11)$$

The eccentricity eigenfrequencies, g_i , are the roots of the determinant

$$\begin{vmatrix} A_{11} - g & A_{12} & \cdots & A_{1n} \\ A_{21} & A_{22} - g & \cdots & A_{2n} \\ \vdots & \vdots & \ddots & \vdots \\ A_{n1} & \cdots & \cdots & A_{nn} - g \end{vmatrix} = 0 \quad (12)$$

while the inclination eigenfrequencies, f_i , are the roots of a similar determinant involving the elements B_{jk} . The coefficients e_{ji} are components of the i th eigenvector of the matrix A , with corresponding eigenvalue g_i :

$$\sum_{k=1}^n A_{jk} e_{ki} = g_i e_{ji}, \quad (j = 1, 2, \dots, n) \quad (13)$$

Similarly, the coefficients I_{ji} ($j = 1, 2, \dots, n$) are components of the i th eigenvector of the matrix B , corresponding to the eigenvalue f_i . A complete solution of this form involves a total of $4n$ arbitrary constants—the magnitudes of the $2n$ eigenvectors, and the corresponding phases, β_i or γ_i , which, in turn, are determined by a complete set of $4n$ initial conditions (e_j , $\tilde{\omega}_j$, I_j , Ω_j ; $j = 1, 2, \dots, n$) at some epoch. Note that the solutions for the eccentricity/pericentre variations are completely decoupled from the inclination/node solutions, thus considerably simplifying the analysis.

There are two circumstances in which the variations of e and $\tilde{\omega}$ (or I and Ω) for a particular satellite are relatively simple. In the first of these two cases, the planetary term in equations (2) and (3), involving J_2 and J_4 , are much larger than the satellite terms. Since all of the Laplace coefficients are comparable in size, it follows that the off-diagonal matrix elements A_{jk} ($k \neq j$) are much smaller than the diagonal elements A_{jj} , so that the eigenfrequencies $g_i \approx A_{ii}$ and the eigenvectors have only small off-diagonal components e_{ji} ($j \neq i$). As a result, the eccentricities are essentially constant and the pericentre precession rates, $\dot{\tilde{\omega}}_j$, are equal to the eigenfrequencies g_j . This is true for all of the inner satellites of Jupiter and Saturn, except Rhea¹⁷, because of these planets' large oblatenesses. In this case, $\dot{\tilde{\omega}}_j \approx g_j \approx A_{jj}$, and $\dot{\Omega}_j \approx f_j \approx B_{jj} \approx -A_{jj}$, so that the nodal precession rate is equal, but of opposite sign, to the pericentre precession rate.

In the second case, the mass of one satellite (j , say) is sufficiently small that its perturbations on the other satellites may be neglected. In consequence, all off-diagonal terms in the j th column of matrix A may be set to zero, and the j th eigenfrequency is again given by $g_j = A_{jj}$ (or $f_j = B_{jj}$). The remaining $n-1$ eigenfrequencies are not, however, necessarily close to the diagonal terms. The eccentricity of satellite j is usually considered as comprising a 'free' component, of frequency g_j , and several 'forced' components, the latter being due to the perturbations by the other satellites. In situations where the free eccentricity is much larger than any of the forced components, the

total eccentricity is again virtually constant and the pericentre precession rate is g_j . Examples of this case are provided by the asteroids, for which the (derived) free eccentricities and inclinations are known as 'proper' elements, and the forced components due to the planets are typically ~30% of the free components.

Neither of these two situations applies in the uranian satellite system: Uranus' J_2 is comparatively small¹⁸, and all five satellites have similar masses. Rather, the uranian system is likely to be more closely analogous to the planetary system, for which J_2 (Sun)=0, and in which all eccentricities and inclinations experience significant secular variations about their mean values. Large variations in e and I , and in $\dot{\omega}$ and $\dot{\Omega}$, are to be expected for each of these satellites, on timescales of decades to centuries. A special case is Miranda, whose proximity to Uranus and relatively small mass are reminiscent of both cases discussed above, and which shows an anomalously large 'free' inclination (see Table 2).

Satellite masses

Because of the lack of orbit-orbit resonances in the uranian satellite system, the mutual gravitational perturbations of the satellites' mean longitudes are small and the satellite masses cannot be determined by methods that have proved useful for many jovian and saturnian satellites. The masses of the uranian satellites are, at present, determined from observations of the motions of their pericentres. Harris⁵, Dunham⁶ and, more recently, Veillet⁴ assume that the forced eccentricities of the uranian satellites are negligible compared with their free eccentricities, and that the pericentre precession rates are constant and given by

$$\dot{\omega}_j = A_{jj} \quad (14)$$

Since all the a_j and n_j values are easily determined, the only significant unknowns in equation (14) are J_2 and the five satellite masses. Before the discovery of the narrow uranian rings, J_2 was not known with useful accuracy and thus Harris and Dunham were able to obtain only poor estimates of the satellite masses. However, the uncertainty in J_2 is now effectively negligible¹⁸ and Veillet has used this fact and his own observational programme to derive the satellite masses from the five observed precession rates (see Tables 1, 2). For Titania and Oberon, in particular, the quoted uncertainties in the masses are smaller than 12%. In Table 2, the observed precession rates, $\dot{\omega}_{\text{obs}}$, are also compared with those that would be produced by the planet alone, $\dot{\omega}_p$. We see that the pericentre rates of Oberon, Titania and even Umbriel are largely determined by the mutual perturbations.

Since the coefficients $b_{3/2}^{(1)}$ and $b_{3/2}^{(2)}$ in equations (2-5) have comparable magnitudes, it follows that if the satellite masses are sufficient to increase significantly $\dot{\omega}_{\text{obs}}$ above $\dot{\omega}_p$, then the off-diagonal terms, A_{jk} and B_{jk} , must make significant contributions to the eigenfrequencies, thus rendering equation (14) inap-

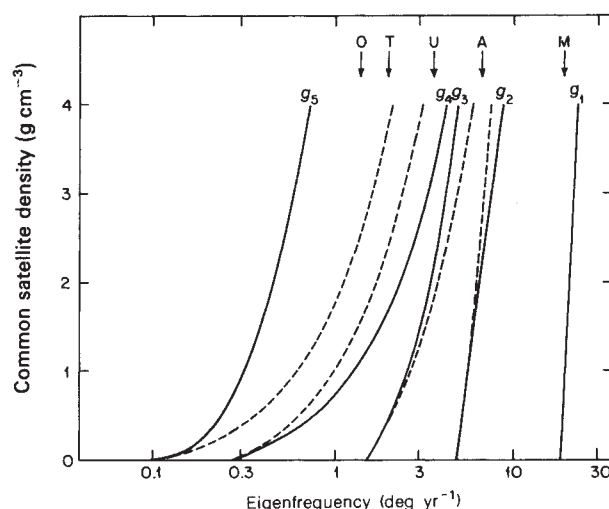


Fig. 1 The solid lines show the variations of the eccentricity eigenfrequencies with the common density of the Uranian satellites. The broken lines are the frequencies associated with the principal diagonal terms of the eigenfrequency matrix. M, A, U, T, and O denote the pericentre precession rates observed by Veillet⁴ for, respectively, Miranda, Ariel, Umbriel, Titania and Oberon.

plicable. It also follows that, if the initial eccentricities of the satellites have comparable magnitudes, then the forced eccentricities are not negligible and e_j and $\dot{\omega}_j$ must suffer significant periodic variations. Such variations have been neglected in all published analyses of the observational data although their potential importance was mentioned by Greenberg in 1975 (ref. 17).

To illustrate the above statements, we consider an heuristic model in which the satellites have a common density ρ . If the off-diagonal terms, A_{jk} , in equation (12) are arbitrarily set to zero, then the forced eccentricities are also zero and the eigenfrequencies are given by the terms, A_{jj} , on the principal diagonal. Figure 1 shows these frequencies as a function of increasing density ρ (the dashed curves) compared with actual eigenfrequencies obtained by solving the full equation (the solid curves). The numbering of the five eigenmodes is chosen so that $g_i \rightarrow A_{ii}$ as $\rho \rightarrow 0$. For the first three modes, the true eigenfrequencies differ only slightly from the dashed curves for all plausible densities. The remaining two eigenmodes, however, which largely reflect interactions between the outer satellites, Titania and Oberon, deviate significantly from the dashed curves for densities as small as 0.5 g cm^{-3} . It is clear then that, at least for these two satellites, equation (14) must be seriously in error.

Table 2 Orbital elements, precession rates and eccentricity damping timescales

<i>j</i>	Satellite	<i>a</i> [*] (km)	<i>e</i>	$\dot{\omega}_0$ [†] (deg)	<i>I</i> (deg)	Ω_0 [†] (deg)	$\dot{\omega}_{\text{obs}}$ (deg yr ⁻¹)	$\dot{\omega}_p$ [*] (deg yr ⁻¹)	<i>T_e</i> / <i>Q</i> [‡] (Myr)
1	Miranda	129775	0.0027 ± 0.0006	111 ± 14	4.22 ± 0.16	21 ± 5	19.8 ± 0.2	19.04	2
2	Ariel	190822	0.0034 ± 0.0003	120 ± 5	0.31 ± 0.11	263 ± 21	6.8 ± 0.3	4.94	0.5
3	Umbriel	265832	0.0050 ± 0.0003	193 ± 3	0.36 ± 0.08	279 ± 12	3.6 ± 0.2	1.55	8
4	Titania	436035	0.0022 ± 0.0001	147 ± 3	0.142 ± 0.031	311 ± 11	2.0 ± 0.1	0.27	50
5	Oberon	583117	0.0008 ± 0.0001	212 ± 7	0.101 ± 0.024	234 ± 16	1.4 ± 0.2	0.10	300

* Calculated from the observed orbital periods, with $GM = 5.784184 \times 10^6 \text{ km}^3 \text{ s}^{-2}$, $J_2 = 3.3450 \times 10^{-3}$, $J_4 = -3.21 \times 10^{-5}$ and $R_p = 26,200 \text{ km}$ (ref. 18). Note that the published value of J_2 has been adjusted to Veillet's determination of $M_{\odot}/M = 22,945$.

† At epoch 0 January 1950, 12 h UT. e , I , $\dot{\omega}_0$, Ω_0 and the observed precession rates, $\dot{\omega}_{\text{obs}}$, are from ref. 4.

‡ The calculated tidal damping timescales, T_e , assume that the satellites are icy and have rigidities of $4 \times 10^{10} \text{ dyn cm}^{-2}$ and densities of 1.3 g cm^{-3} . Q is the tidal dissipation function and probably has a value $\sim 10^2$ (ref. 26).

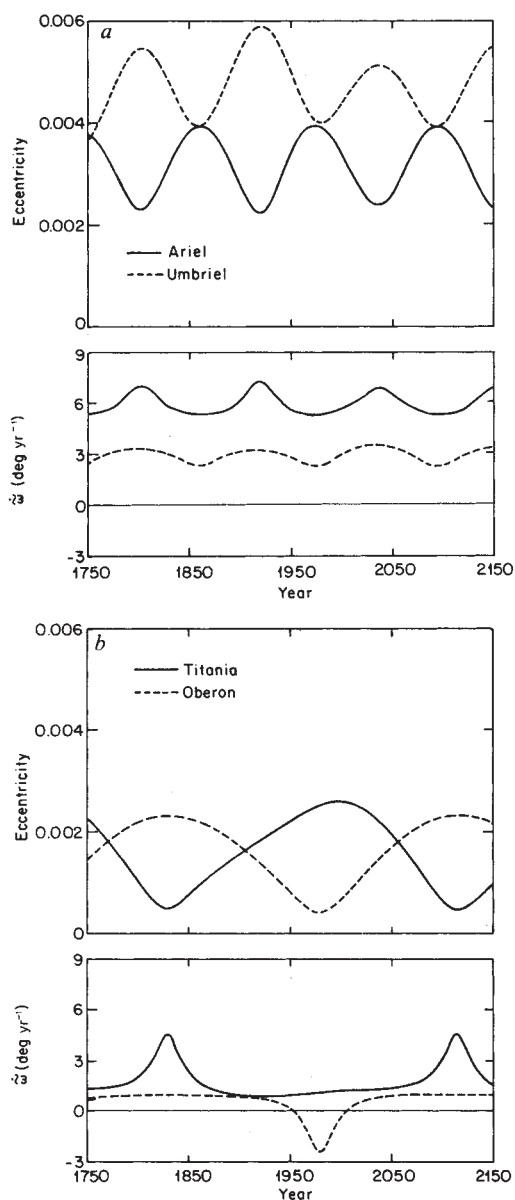


Fig. 2 Possible variations of the eccentricities and the pericentre precession rates of Ariel and Umbriel (a) and Titania and Oberon (b). We assume that the uranian satellites have a common density of 1.3 g cm^{-3} . The initial conditions are the orbital elements listed by Veillet⁴ for the epoch 1950.0 (see Table 2).

Using masses for a nominal common satellite density of 1.3 g cm^{-3} (see Table 1), we have also calculated the normalized eccentricity and inclination eigenvectors shown in Table 3. These quantities together with the orbital elements determined by Veillet for epoch 1950.0 (see Table 2), which we use as a set of initial conditions, enable us to determine the amplitudes and the phases of the 10 eigenmodes and hence the variations of the orbital elements with time. Our results are shown in Figs 2–4. These should not be taken literally as representing 'the' variation of the uranian satellites' orbital elements, but rather as a typical example of many possible solutions. Not only is our assumption of a uniform density of 1.3 g cm^{-3} completely arbitrary (although plausible), but Veillet's elements were derived on the incorrect assumption that e and I are independent of time.

In all of these calculations, we have used the best values currently available¹⁸ for Uranus' J_2 and J_4 , adjusted to Veillet's determination of M . The semimajor axes of the satellites were

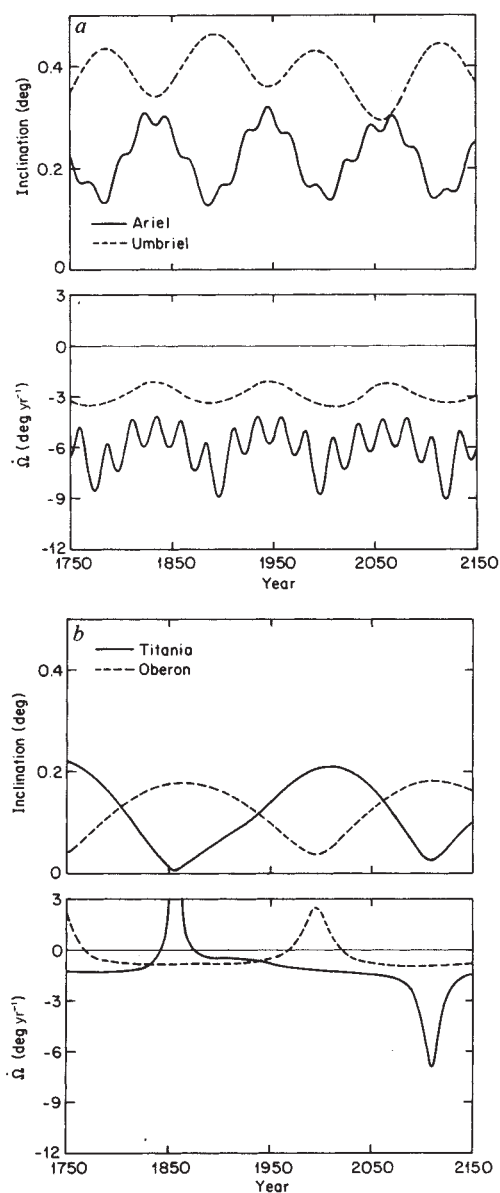


Fig. 3 Possible variations of the inclinations and the nodal precession rates of Ariel and Umbriel (a) and Titania and Oberon (b). We assume that the uranian satellites have a common density of 1.3 g cm^{-3} . The initial conditions are the orbital elements listed by Veillet⁴ for the epoch 1950.0 (see Table 2).

re-calculated from the observed orbital periods, using the same values for M , J_2 , and J_4 .

Density of Ariel

Veillet⁴ reports that the orbital inclination of Miranda is $4.22^\circ \pm 0.16^\circ$, in good agreement with the value of $4.0^\circ \pm 0.3^\circ$ obtained by Greenberg and Whitaker^{19,20}. The forced inclination components are negligible compared with this anomalously high value and $\dot{\Omega}$ is, therefore, effectively constant, as shown in Fig. 4. This fact, combined with the near-equality of f_1 and B_{11} (compare with g_1 in Fig. 1), implies that the difference between the observed value of Miranda's nodal precession rate, $\dot{\Omega}_1$, and that due to the planetary oblateness alone, $\dot{\Omega}_p$ is related to the masses of the other satellites by

$$\dot{\Omega}_1 - \dot{\Omega}_p \approx f_1 - \dot{\Omega}_p \approx -\frac{1}{4} n_1 \sum_{k=2}^5 \frac{m_k}{M} \alpha_{jk} \bar{\alpha}_{jk} b_{3/2}^{(1)}(\alpha_{jk}) \quad (15)$$

Using the radii of Brown^{2,3} and assuming that the four satellites

have a common density, $\rho_A \text{ g cm}^{-3}$, this difference can be written

$$\dot{\Omega}_1 - \dot{\Omega}_p = -1.20 \rho_A \text{ deg yr}^{-1} \quad (16)$$

Since Ariel accounts for $\sim 80\%$ of the above nodal rate difference, ρ_A is effectively the density of Ariel. $\dot{\Omega}_p = -18.99 \text{ deg yr}^{-1}$, allowing for Miranda's inclination of 4.2° (ref. 4). If $\dot{\Omega}_1 = -19.76 \pm 0.22 \text{ deg yr}^{-1}$, as reported by Veillet⁴, then, allowing for the uncertainties in the satellite radii, we obtain:

$$\rho_A = 0.64 \pm 0.26 \text{ g cm}^{-3} \quad (17)$$

This value is quite different from that calculated by Veillet²¹, largely because he used an inappropriate value for the radius of Uranus (it is only the product $R^2 J_2$ that is accurately known from studies of the rings). If the densities of the outer satellites are greater than that of Ariel, or if unseen satellites of significant mass exist anywhere in the uranian system, then the density of Ariel must be even less than the remarkably low value given above. This low density is inconsistent with that of a satellite with a composition of rock and water-ice.

Greenberg and Whitaker¹⁹, on the other hand, obtained a nodal precession rate for Miranda of $-21.6 \pm 0.7 \text{ deg yr}^{-1}$, implying a value for ρ_A of $2.2 \pm 0.9 \text{ g cm}^{-3}$. These apparently inconsistent results are disturbing, given the expected constancy of I_1 and $\dot{\Omega}_1$, but the greatly enlarged data set used by Veillet should lead to his value being preferred at present. At any rate, it is apparent that an accurate future determination of Miranda's nodal precession rate would yield an important constraint on Ariel's density.

Forced eccentricities

Tidal dissipation due to the tide raised by a planet on a satellite tends to circularize the satellite's orbit, while heating the satellite⁸. In the absence of eccentricity forcing, the orbital eccentricities of Miranda and Ariel would probably decay on timescales significantly less than the age of the Solar System (see Table 2). Secular perturbations, on the other hand, result in an exchange of angular momentum between the inner and outer satellites on much shorter timescales (10–1,000 yr), and are thus likely to considerably extend the eccentricity-decay times calculated in Table 2. Essentially, the process of tidal dissipation must now be thought of as damping entire eigenmodes, rather than individual satellite eccentricities. Naturally, those modes which have their largest amplitudes for the inner satellites, that is modes 1 and 2 in Table 3a, will be damped most rapidly, while modes 4 and 5, involving principally Titania and Oberon, will be damped much more slowly.

An examination of the observed eccentricities of Miranda and Ariel, however, suggests that even modes 1 and 2 have not yet been completely damped. Based on the eigenmode solution in Table 3, we find the 'forced' component of Miranda's eccentricity (due principally to Ariel) to be ~ 0.0003 , or only about 10% of the observed value, while the forced component of Ariel's eccentricity (due principally to Umbriel) is ≈ 0.0008 , $\sim 25\%$ of the observed value. (The term 'forced' is used loosely to refer to that component of the eccentricity which is not due to the eigenmode most closely associated with the satellite in question). This conclusion is supported by the observation that Miranda and Ariel exhibit pericentre precession rates close to the calculated eigenfrequencies for modes 1 and 2, respectively (see Tables 2, 3 and Fig. 1).

Thus, we cannot account for the observed eccentricities of Miranda and Ariel purely in terms of forcing by the outer satellites. However, since Veillet's observational data need to be re-reduced in the light of our findings on the variability of the orbital elements, the latter conclusion may be premature. We note that the osculating elements of Miranda obtained by Veillet from data taken on eight nearly consecutive nights in April 1981 give an eccentricity of 0.0007 ± 0.0004 (ref. 21). This low value is consistent with what we would expect if tidal

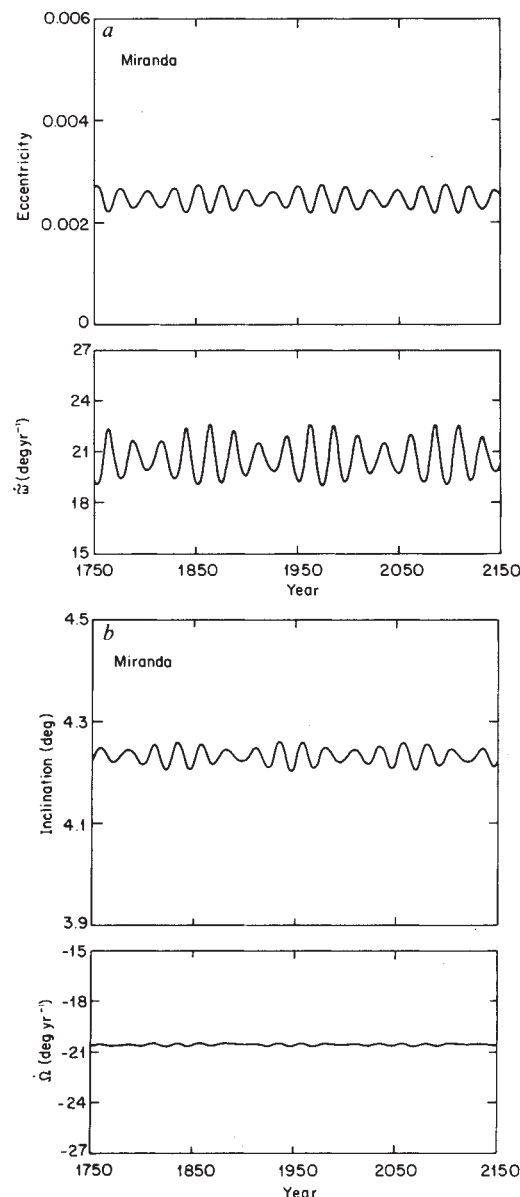


Fig. 4 Possible variations of the eccentricity (a) and the inclination (b) of Miranda and the corresponding variations in the precession rates, $\dot{\omega}$ and $\dot{\Omega}$. We assume that the uranian satellites have a common density of 1.3 g cm^{-3} . The initial conditions are the orbital elements listed by Veillet⁴ for the epoch 1950.0 (see Table 2).

dissipation had entirely damped out the first eccentricity eigenmode, but not the second.

If the large amplitudes of the first two eigenmodes are confirmed by subsequent studies, then maintenance mechanisms outside those provided by low-order secular perturbation theory must be sought. Perturbation terms of second-order in the masses will produce variations in e and I on timescales $\Gamma \sim T/(m/M)^2$, where T is the characteristic orbital period. For the uranian satellites, $\Gamma \sim 10^8 \text{ yr}$. If Γ is less than the eccentricity damping timescales, it is conceivable that these second-order perturbations may result in an exchange of angular momentum between the inner and the outer satellites sufficient to maintain the eccentricities of the inner satellites. This possibility will be investigated in the near future, by means of very long-term numerical integrations of test satellite systems (A. Milani and A. M. Nobili, personal communication). Another possibility is that an expansion of the orbits of Miranda and Ariel due to tidal dissipation in the planet has resulted in the comparatively

Table 3 Eccentricity and inclination eigenvectors for a common satellite density of 1.3 g cm^{-3}

a Normalized eccentricity eigenvectors						
Satellite	<i>j</i>	Mode 1	Mode 2	Mode 3	Mode 4	Mode 5
Miranda	1	0.9999	0.0674	0.0146	0.0036	0.0026
Ariel	2	-0.0031	0.9678	0.1688	0.0347	0.0216
Umbriel	3	-0.0002	-0.2424	0.9841	0.1744	0.0955
Titania	4	0.0000	-0.0024	-0.0521	0.8287	0.6098
Oberon	5	0.0000	-0.0004	0.0060	-0.5307	0.7865
Eigenfrequency (deg yr^{-1})		20.612	5.951	2.878	1.610	0.352
b Normalized inclination eigenvectors						
Satellite	<i>j</i>	Mode 1	Mode 2	Mode 3	Mode 4	Mode 5
Miranda	1	0.9999	0.0836	0.0225	0.0082	0.0070
Ariel	2	-0.0039	0.9553	0.1979	0.0599	0.0413
Umbriel	3	-0.0003	-0.2837	0.9769	0.2488	0.1423
Titania	4	0.0000	-0.0053	-0.0770	0.7972	0.6278
Oberon	5	0.0000	-0.0013	0.0097	-0.5468	0.7641
Eigenfrequency (deg yr^{-1})		-20.560	-6.005	-2.840	-1.696	-0.249

recent passage of these satellites through orbit-orbit resonances¹⁴. Passage through resonance may have resulted in the excitation of both a high eccentricity and a high inclination, but whereas the eccentricity would subsequently be damped by tidal dissipation, the high inclination would remain and would testify to the resonance passage.

Discussion

Because of the neglect of secular variations in all published analyses of the orbits and masses of the uranian satellites, including that of Veillet⁴, the results obtained from these investigations must be treated with caution. The masses in particular, derived from an inappropriate expression for pericentre precession, may well be considerably in error. Unfortunately, it does not appear possible to correct these omissions and re-derive better values for the satellite masses without a reconsideration of all the available data. Such an investigation is beyond the scope of this paper. A thorough re-analysis of the uranian satellites' orbital elements, taking into account the present results, would probably start with the establishment of osculating elements from short (≤ 5 yr) spans of data, and then attempt to model the longer-term variations in these osculating elements in terms of the theory of secular perturbations. Hints of such variations are already present in the large differences between some of the orbital elements derived by Harris in 1950, Dunham in 1970 and Veillet in 1983. Apart from yielding an improved set of masses, such an investigation may be expected to shed light on several other outstanding problems. These include: (1) a discrepancy of $\sim 0.2^\circ$ between the pole direction of Uranus deduced from stellar occultation studies of the rings^{18,22} and that obtained by Veillet⁴; (2) a discrepancy between the observed

period of the Miranda-Ariel-Umbriel longitude variation and that deduced from Veillet's masses²³; and (3) the question of whether any 'secular librations' exist among the satellites, as suggested by Greenberg¹⁷. The results shown in Figs 2 and 3 and Table 3 indicate that Titania and Oberon could well be involved in such a libration, involving the arguments $\tilde{\omega}_4 - \tilde{\omega}_5$ and/or $\Omega_4 - \Omega_5$.

Although it does not seem possible, at present, to ascribe the apparently undamped eccentricities of Miranda and Ariel to secular perturbations alone, it is clear that a more realistic estimate of tidal damping timescales for the uranian satellites must take such perturbations into account. An investigation of this matter will form the subject of a future paper.

Finally, we note that the encounter with Uranus of the Voyager 2 spacecraft on 24 January 1986 should provide several pieces of information relevant to uranian satellite dynamics. Most important would be direct measurements of the satellite masses, which now must be considered as unknown. Only Miranda is to be approached relatively closely by the spacecraft, but all necessary steps should be taken to maximize the chances of successful mass determinations for the other four satellites, especially Ariel. Of secondary importance, but also valuable, would be the determination of a complete set of osculating orbital elements for the satellites, based on far-encounter and near-encounter imaging. In particular, an accurate Voyager determination of Miranda's node, combined with extensive ground-based observations of this satellite carried out over the past 9 yr (refs 21, 24, 25) should lead to an independent estimate of Ariel's density through equation (15).

We thank D. J. Stevenson, R. Hamilton Brown and W. Reid Thompson for useful discussions. This research was supported by NASA grants.

Received 29 July; accepted 31 October 1985.

1. Cruikshank, D. P. in *Uranus and the Outer Planets* (ed. Hunt, G.) 193-210 (Cambridge University Press, 1982).
2. Brown, R. H., Cruikshank, D. P. & Morrison, D. *Nature* **300**, 423-425 (1983).
3. Brown, R. H. & Clark, R. N. *Icarus* (in the press).
4. Veillet, C. thesis Univ. Paris 6 (1983).
5. Harris, D. L. thesis, Univ. Chicago (1949).
6. Dunham, D. W. thesis, Yale Univ. (1971).
7. Brown R. H. in *Uranus and Neptune* (ed. Bergstrahl, J. T.) 437-461 (NASA Con. Pub. 2330, 1984).
8. Peale, S. J., Cassen, P. & Reynolds, R. T. *Science* **203**, 892-894 (1979).
9. Morabito, J., Synnott, S. D., Kupferman, P. W. & Collins, S. A. *Science* **204**, 972 (1979).
10. Cassen, P., Peale, S. J. & Reynolds, R. T. *Geophys. Res. Lett.* **7**, 963-970 (1980).
11. Cassen, P., Peale, S. J. & Reynolds, R. T. *Geophys. Res. Lett.* **6**, 731-734 (1979).
12. Squyres, S. W., Reynolds, R. T., Cassen, P. & Peale, S. J. *Nature* **301**, 225-226 (1983).
13. Squyres, S. W., Reynolds, R. T., Cassen, P. & Peale, S. J. *Icarus* **53**, 319-331 (1983).

14. Dermott, S. F. in *Uranus and Neptune* (ed. Bergstrahl, J. T.) 377-404 (NASA Conf. Publ. 2330, 1984).
15. Squyres, S. W., Reynolds, R. T. & Lissauer, J. J. *Icarus* **61**, 218-223 (1985).
16. Brouwer, D. & Clemence, G. M. *Methods of Celestial Mechanics* (Academic, New York, 1961).
17. Greenberg, R. *Mon. Not. R. astr. Soc.* **170**, 295-303 (1975); in *Uranus and Neptune* (ed. Bergstrahl, J. T.) 463-480 (NASA Conf. Publ. 2330, 1984).
18. French, R. G., Elliot, J. L. & Levine, S. E. *Icarus* (submitted).
19. Greenberg, R. & Whitaker, E. A. *Bull. Am. astr. Soc.* **6**, 207 (1974).
20. Whitaker, E. A. & Greenberg, R. *Mon. Not. R. astr. Soc.* **165**, 15p-18p (1973).
21. Veillet, C. *Astr. Astrophys.* **118**, 211-216 (1983).
22. Elliot, J. L. & Nicholson, P. D. in *Planetary Rings* (eds Greenberg, R. & Brahic, A.) 25-72 (University of Arizona Press, Tucson, 1984).
23. Lazzaro, D., Ferraz-Mello, S. & Veillet, C. *Astr. Astrophys.* **140**, 33-38 (1984).
24. Veillet, C., & Ratier, G. *Astr. Astrophys.* **89**, 342-344 (1980).
25. Veillet, C. *Astr. Astrophys.* **98**, 218-222 (1981).
26. Peale, S. J., Cassen, P. & Reynolds, R. T. *Icarus* **43**, 65-72 (1980).

Requirement of stereospecific alignments for initiation from the simian virus 40 early promoter

Keikichi Takahashi*, Marc Vigneron*, Hans Matthes, Alan Wildeman*,
M. Zenke* & Pierre Chambon

Laboratoire de Génétique Moléculaire des Eucaryotes, CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique, INSERM, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg 67085 Cédex, France

The distance between the simian virus 40 early promoter elements has been altered by inserting either odd or even multiples of half a DNA turn. There are marked differences in the in vivo effects of these two types of insertions on initiation of transcription from this promoter.

DISSECTION *in vivo* and *in vitro* of the simian virus 40 (SV40) early promoter region (see Fig. 1) has shown that it contains two overlapping promoters for RNA polymerase B (II), the early-early (EE) and late-early (LE) promoters (see refs 1–5 and refs therein). Accurate initiation of RNA synthesis at the early-early start sites (EES) requires the TATA box element, whereas the 21-base pair (bp) repeat region and the enhancer are both essential for efficient transcription. Efficient initiation of transcription from the late-early start sites (LES) also requires the presence of the 21-bp repeat region and of the enhancer, and occurs in the apparent absence of a TATA box. Six G-C motifs (Fig. 1; I–VI) are involved in the function of the 21-bp repeat region, but they do not contribute equally to stimulation of transcription from the EES and LES, G-C motifs I–III being the most important for initiation from EES, whereas initiation from LES is more dependent on G-C motifs IV–VI^{4,5}. A systematic mutational dissection of the enhancer region has revealed that several sequence motifs are indispensable to its function, and that the same sequences are responsible for enhancement of transcription from both the EES and the LES⁶.

In vitro transcription studies have shown that different *trans*-acting factors bind specifically to these *cis*-acting regulatory sequences. The TATA box factor binds to the TATA box to form a stable complex; this is a prerequisite for specific initiation of transcription^{7,8}. The transcription factor Sp1 binds specifically to the 21-bp repeat region and stimulates transcription from the EES and LES^{4,9–11}. Different *trans*-acting factor(s) bind(s) to the enhancer and stimulate(s) transcription from both the SV40 early and heterologous promoter elements^{12–15}. Moreover, footprinting experiments have shown that binding *in vitro* of the Sp1 factor^{4,10,11} and enhancer¹⁶ factor(s) to the 21-bp repeat and the enhancer regions, respectively, is severely impaired by mutations known to be detrimental to the *in vivo* function of these promoter elements.

Therefore, at least three different proteins (or sets of proteins), interacting with three different promoter elements, are required to allow RNA polymerase B to initiate transcription from the SV40 early promoter. However, both the 21-bp repeat element and the enhancer can operate when in an inverted orientation (refs 4–6 and refs therein), although they do not exhibit any obvious symmetry in their DNA sequence. In addition, the enhancer can stimulate transcription to some extent when inserted some distance away^{17–19}. Thus, rather than stimulating transcription by interacting with each other and/or with RNA

polymerase, the factors that bind to the 21-bp repeat and enhancer regions could induce a local perturbation in the structure of the DNA helix, which could be propagated close to the start sites, where it would facilitate initiation of transcription by RNA polymerase B. We have altered the distance between the three SV40 early promoter elements by inserting either odd or even multiples of half a turn of the DNA helix (DNA turn), and demonstrate here that there are marked differences in the *in vivo* effects of these two types of insertions on initiation from this promoter, which strongly suggests that protein–protein interactions are involved in activation of transcription initiation from an RNA polymerase B promoter.

Construction and assay of spacing mutants

All spacing mutant recombinants are similar to pDHB3.SV1 (ref. 5) and pSEGO (ref. 4) in that they contain the SV40 early promoter region (from *Hpa*II to *Hind*III; Fig. 1) inserted upstream from a promoter-less rabbit β -globin gene (position –9 to +1,650) and a complete rabbit β -globin gene (including its promoter region), acting as an internal 'reference' gene. The pS series contains 4–29-bp inserts at the *Sal*I site (position 32) between the 21-bp repeat and the TATA box region of pSO. The pSE series harbours 5–25-bp inserts at the *Bam*HI site (position 101) between the enhancer and 21-bp repeat region of pSEGO (similar to pSO, but containing a single 72-bp sequence). In the pSR series, inserts of 4–21 bp are introduced between the TATA box and the inverted 21-bp repeat region (from position 40 to 103) of pSRO (otherwise identical to pSO). The pSR series also contains 4–7-bp deletion mutants (Fig. 1). For most spacer lengths, we constructed two recombinants, A and B, with different inserted sequences (usually the same sequence in both orientations).

After transfection of the recombinants into HeLa cells, RNA initiated from the SV40 early promoter was measured by quantitative *S*₁ nuclease mapping using two probes as described previously^{4,5}. The amount of RNA initiated from the reference β -globin gene (GLOB band in Figs 2–4) and the total amount of RNA initiated from the SV40 early promoter region (EP band in Figs 2–4) was determined with probe A. Probe B was used to accurately map and quantitate RNA initiated from the EES and LES of the SV40 early promoter (Figs 1–4; under the present stringent *S*₁ nuclease conditions, the bands corresponding to LES2 and LES3 were not resolved and no attempt was made to measure initiation from LES1—see refs 1 and 5 for a discussion of these points).

DNA turn-dependent insertions

Between the enhancer and 21-bp repeat region. Introduction of 10 bp and 21 bp, both near-integral multiples of the 10.5 bp per turn of B-DNA *in vitro*²⁰, between the 21-bp repeat region and the enhancer of pSEGO, causes an ~50% decrease in RNA

* Present addresses: Medical Institute for Bioregulation, Kyushu University, Maedashi 3-1-1, Higashi-ku, Fukuoka 812, Japan (K.T.); Department of Virology, The Weizmann Institute for Science, Rehovot, Israel (M.V.); Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada N1G 2W1 (A.W.); European Molecular Biology Laboratory, Postfach 102209, D-6900 Heidelberg, FRG (M.Z.).

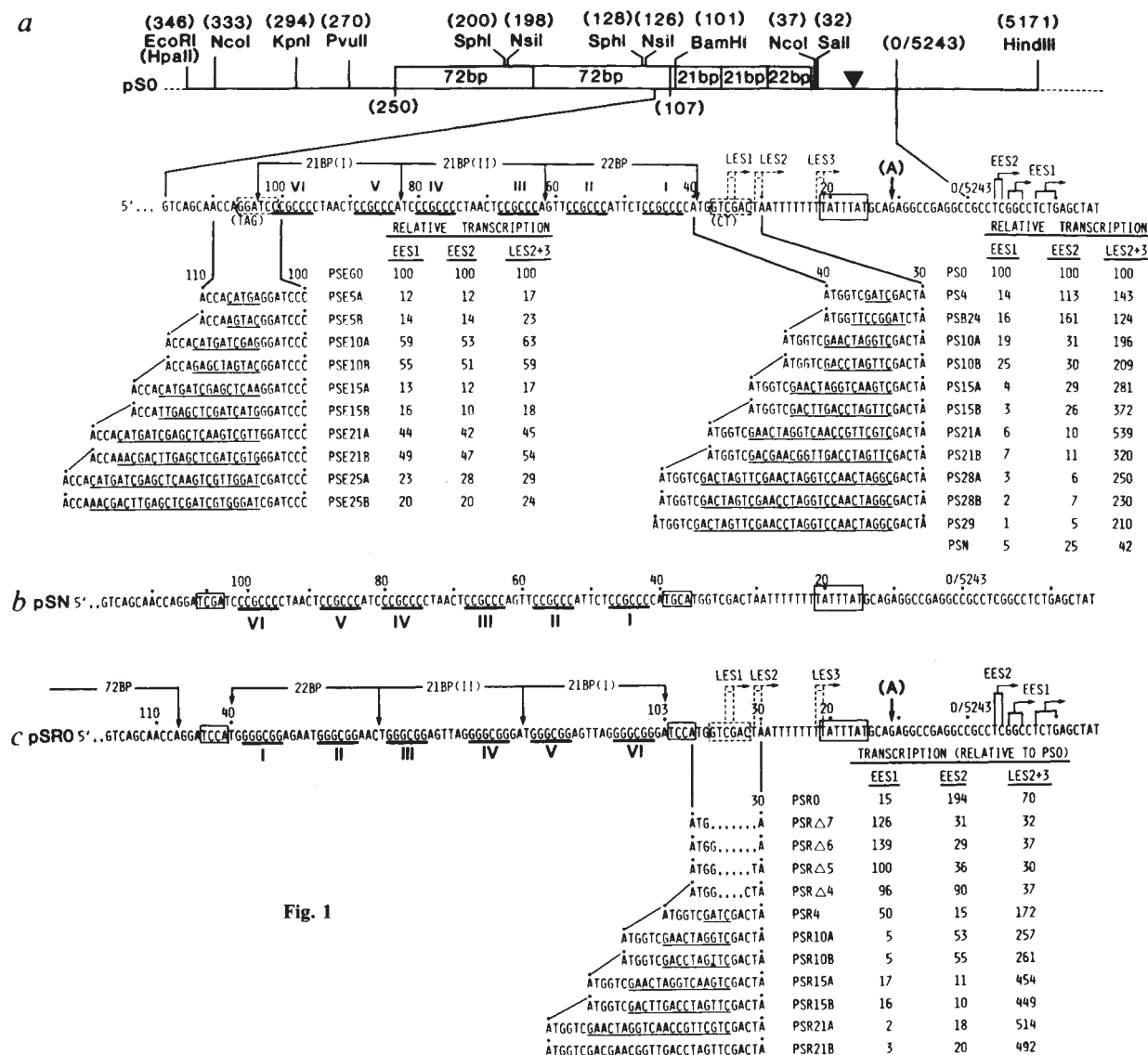


Fig. 1

initiated from all EES and LES (pSE10 and 21 mutants; Figs 1, 2, 5). In marked contrast, alterations of the distance between these two promoter elements by odd multiples of half a DNA turn result in dramatic 80–90% decreases in transcription initiated from all start sites (pSE5, 15 and 25 mutants; Figs 1, 2, 5). This is well illustrated by the 'cyclic' variations in the total amount of RNA initiated from the SV40 early promoter region estimated from the intensity of the band EP in Fig. 2. As the EE and LE promoters share a common element—the 21-bp repeat region—these results indicate that efficient transcription from both the EES and LES requires stereospecific alignments between some elements of the enhancer and of the 21-bp repeat region (see below for further discussion). The present results also confirm our previous conclusion¹ that the enhancer is required for activating transcription from both the SV40 EE and LE promoters.

Several lines of evidence support the conclusion that the observed variations reflect modifications of the effect of the enhancer and not of the activity of a putative upstream element similar to the 21-bp repeat region, which would also be contained in the enhancer sequence. First, no mutations within the enhancer have been found that affect its activity specifically when it is in close apposition to the 21-bp repeat region, but not when it is moved far away⁶. Second, the level of all RNA species initiated downstream from the enhancer varies in parallel, which is in marked contrast to the differential variations observed with

insertions between the 21-bp repeat region and the TATA box (see below). Third, there is no rapid overall decrease of RNA synthesis as the length of the 'even inserts' is increased and no decrease at all with 'odd inserts', which again is in marked contrast to the variations observed in either the pS or pSR series (see below). The last observation probably reflects the increasing possibility of achieving a proper alignment as the length of the odd inserts increases.

The sequence element(s) which, within the enhancer domains, is (are) responsible for this DNA turn-dependence of activation of transcription is (are) unknown. The SV40 enhancer is composed of at least two domains, A and B, which are very inefficient by themselves but, when juxtaposed, stimulate transcription from the early promoter by three orders of magnitude⁶. In an enhancer containing a single 72-bp sequence, domain A, which is centred on the *Sph*I site, spans the 3' moiety of the 72-bp sequence, whereas domain B roughly spans the 5' moiety of the 72-bp sequence and elements located further upstream up to approximately the *Pvu*II site (ref. 6; see also Fig. 1). Inserting fragments of various lengths (4–50 bp) between domains A and B does not markedly affect the enhancer activity⁶, indicating that there is apparently no stringent position requirement between the two domains nor between domain B and the 21-bp repeat region. (However, longer insertions result in a strong decrease of enhancer activity; see ref. 6.) It appears, therefore, that the cyclic variations in RNA synthesis observed in the pSE

Fig. 1 (Opposite) General organization and sequences of the SV40 early promoter region and the pS, pSE and pSR mutant series. *a*, Organization of SV40 early promoter region in recombinant pSO. Some key natural or engineered (*Bam*HI, *Sal*I) restriction sites are indicated with their position (BBB system; see ref. 29) in parentheses. **▼**, Location of the TATA box; the sequence of pSO (noncoding early strand) between coordinates 5,227 and 117 is shown. The SV40 wild-type bases mutated during the creation of the *Bam*HI (101) and *Sal*I (32) sites (dashed boxes; see ref. 4), are indicated in parentheses below the main sequence. The TATA sequence is boxed. The position of the early-early start sites (EES1 and EES2, arrows) and late-early start sites (LES1, LES2 and LES3, dashed arrows) are indicated (see ref. 1 and text). The position of start site (A) corresponding to the G residue at position 11 is indicated (see Fig. 3 and text). The directly repeated 21-bp sequences (1 and 11), and the 22-bp sequences are delimited by vertical, downward-pointing arrows. The six G-C motifs 5' CCGCCC 3' are underlined and identified by roman numerals⁴. The sequences inserted (underlined) in the pSE and pS mutant series are represented on the left- and right-hand sides below the pSO sequence, respectively. The pSE mutant series is derived from pSEGO (ref. 4), which contains a single 72-bp sequence, whereas the pS mutant series is derived from pSO which contains a complete 72-bp repeat (see below). Sequences of pSN (*b*) and pSRO (*c*), in which the 21-bp repeat region is in the natural and reverse orientation, are shown. Boxed sequences at the borders of the 21-bp repeat region correspond to the 4-bp inserts generated during the construction of these recombinants. pSN and pSRO have a complete 72-bp repeat. The sequences deleted or inserted (underlined) in the pSR mutant series are indicated below the pSRO sequence. The relative amount of RNA initiated at EES1, EES2 and LES2+3 after transfection of the various recombinants into HeLa cells was estimated by scanning autoradiograms (similar to those shown in Figs 2-4) from at least three different quantitative S₁ nuclease mapping experiments using different plasmid preparations. After correction for transcription from the reference β -globin gene^{4,5} the results were expressed relative to pSO (for the pS and pSR series) or pSEGO (for the pSE series), taken as 100%.

Methods. Construction of pSO, pSRO and pSN. pSO, which is identical to pSEGO⁴ but contains a complete 72-bp repeat, was constructed by ligating the *Hind*III/*Bam*HI fragment (position 5,171-101, BBB numbering system²⁹) of pSEGO into the equivalent position of pSVTK2 (ref. 4). pSRO and pSN were constructed as follows: pTK4 is similar to pHB4 (ref. 4), but contains a complete 72-bp repeat, a *Sal*I site at position 32 (by exchange of the *Hind*III (5,171)/*Nco*I (37) fragment with that of pSEGO), and lacks the *Sal*I and *Sph*I sites in the pBR322 sequence (destroyed by repairing with Klenow DNA polymerase and blunt-end ligation). pTK4 was digested with *Nco*I (position 37) and *Bam*HI (position 101), and treated with Klenow DNA polymerase in the presence of all four dNTPs. The fragments were ligated back to yield two recombinants (pTK421N and pTK421R) containing the 21-bp region in either orientation between the repaired *Nco*I and *Bam*HI sites. The *Sal*I/*Kpn*I fragments (positions 32 and 294) of these two recombinants were isolated and ligated to pSO digested by *Sal*I and *Kpn*I to yield pSN and pSRO. Construction of mutants with insertions and deletions between the 21-bp repeat region and the TATA box region: Three sets of two overlapping complementary oligonucleotides (10-, 15-, 21-mers) with *Sal*I protruding 5' ends were synthesized by the phosphotriester method as described previously³⁰. The oligonucleotides were phosphorylated, mixed, heated to 60 °C for 30 min and then hybridized at room temperature for 1 h. pSO and pSR DNAs were digested with *Sal*I and ligated to the oligonucleotides to yield the pS (pS10, 15, 21) and pSR (pSR10, 15 and 21) mutant series, respectively, with inserts in either orientation (A and B). pS4 and pSR4 were constructed by blunt-end ligation of pSO and pSR cleaved with *Sal*I and repaired with Klenow DNA polymerase. pSB24 containing a different 4-bp insert was constructed by replacing the *Hind*III/*Nco*I (5,171-37) fragment of pSO with that of pDB3.B24². pS28A, pS28B and pS29 were obtained during the cloning of pS10 (see Fig. 1). Deletion mutants of pSR were prepared as follows: 30 μ g of pSRO was digested with *Sal*I, phenol-chloroform-extracted, precipitated with ethanol, and suspended in 400 μ l S₁ nuclease mixture (30 mM NaOAc pH 4.5, 3 mM ZnSO₄, 400 mM NaCl). 250 units of S₁ nuclease were added, and after 10 min at 25 °C the reaction was stopped by adding 70 μ l of 2 M Tris-HCl pH 8.0, 0.2 M EDTA. After phenol-chloroform extraction, the DNA was precipitated with ethanol and suspended in 30 μ l 10 mM Tris-HCl pH 8.0, 1 mM EDTA. DNA (1 μ g) was treated with Klenow DNA polymerase in the presence of all four dNTPs, then ligated to yield pSR Δ 7-pSR Δ 4 deletion mutants. Construction of mutants with insertions between the 21-bp repeat and the enhancer: The pSE mutant series was constructed by shot-gun ligation of synthetic oligonucleotides³¹. Briefly, eight sets of different overlapping oligonucleotides corresponding to the sequence between the *Bam*HI and *Nsi*I sites (positions 101 and 126) plus additional sequences of 5, 10, 15 and 21 bp at the *Bam*HI extremity were chemically synthesized. The oligonucleotides were phosphorylated at their 5' ends, mixed and hybridized as above, and ligated directly into pSEGO (ref. 4) digested with *Nsi*I and *Bam*HI to yield the pSE mutant series. pSE25 (A and B) were constructed by cleaving pSE21 with *Bam*HI, followed by blunt-end ligation after reparation with Klenow DNA polymerase. Recombinant DNAs were grown and prepared as described previously⁴ and monitored for possible rearrangements by restriction enzyme analysis. Sequence and orientation of the DNA inserts were determined by dideoxynucleotide sequencing of both strands using double-stranded plasmid DNAs as template, and synthetic oligonucleotide primers³².

series reflect the necessity of a stereo-alignment between some elements contained in enhancer domain A and the G-C motifs of the 21-bp repeat region.

Between the 21-bp repeat and TATA box regions. In agreement with our previous results^{4,5}, insertion of DNA segments of increasing length between the 21-bp repeat and the TATA box regions leads to a rapid and drastic decrease in RNA initiated at the major early-early start sites, EES1 (Figs 1, 3, 5). It is, however, remarkable that 10- (pS10A and B) and 21-bp (pS21A and B) inserts are consistently less detrimental to transcription than 4- (pS4 and pSB24) and 15-bp (pS15A and B) inserts, respectively. It is particularly striking that the intensity of the two bands labelled EES2 in Fig. 3 varies in exactly the opposite way (see also Figs 1, 5). These two bands correspond to minor start sites which, contrary to EES1, do not disappear when the TATA box is mutated¹, but are absent when the row of Ts located immediately upstream from the TATA box is mutated (M. Pauly, M. Wintzerith, H.M. and P.C., unpublished results). It is probable, therefore, that this row of Ts acts as a substitute TATA box to direct initiation of transcription at EES2.

We conclude that some upstream elements must be located stereospecifically with respect to a given TATA box for initiation of transcription under its control to occur. To characterize these elements further, we constructed plasmid pSN (Figs 1, 4), which contains a 4-bp insertion at both ends of the 21-bp repeat region, so as to maintain the TATA box and enhancer elements close to their natural steric relationship. The pattern of initiation in pSN (EES2 favoured compared with EES1; Figs 1, 4) is very similar to those of pS4 and pSB24, which possess a single 4-bp insertion between the TATA box and the 21-bp repeat region. This result indicates that it is the steric location, relative to the TATA box, of the 21-bp repeat region rather than that of the enhancer which is responsible for the occurrence of initiation preferentially at EES1 or EES2. However, as expected from the results obtained with the pSE5 mutants, there was a general

decrease in transcription from pSN compared with pS4 and pSB24.

Several lines of evidence support the conclusion that the region at which transcription initiation occurs is primarily determined by the location of the 21-bp repeat region, and that the TATA box element selects the precise site of initiation. Moving the 21-bp repeat region away from the TATA box region by >20 bp results in a dramatic decrease of transcription from both EES1 and EES2 start sites, whereas initiation from the LES is concomitantly increased, indicating that the location of the 21-bp repeat region is not optimal for initiation from LES. Using longer DNA inserts, we found that initiation at LES disappears, whereas new start sites located within a 30-50-bp region downstream from the 21-bp repeat region appear⁴. Note that with insertions of $\leq$ 21 bp there is very little initiation of transcription within the inserted sequence, as the band corresponding to the endpoint of homology between the wild-type probe and RNA transcribed from the spacing mutants is very weak, with the exception of mutants pS28A and B (not shown) and pS29 (Fig. 3, right-hand arrow). Whether the same late-early start sites were used in all insertion mutants cannot be determined from the present experiments, as all start sites in the A+T-rich region (positions 14-31) yield the same LES2+3 bands under the S₁ nuclease conditions used in this study (see above; there is, however, an additional start site A (Fig. 3) which appears to be preferentially used in some mutants). Thus, it is not possible from the present study to determine whether initiation of transcription from the various LES is also DNA turn-dependent.

Insertions and deletions between the inverted 21-bp repeat and TATA box regions. The 21-bp repeat region can stimulate initiation of transcription from the EES and LES, even when inserted in the reverse orientation in the SV40 early promoter region^{2,4,5,21}. The pSR mutant series (Fig. 1) was constructed to determine whether altering the distance between the inverted 21-bp repeat region and the TATA box region would result in

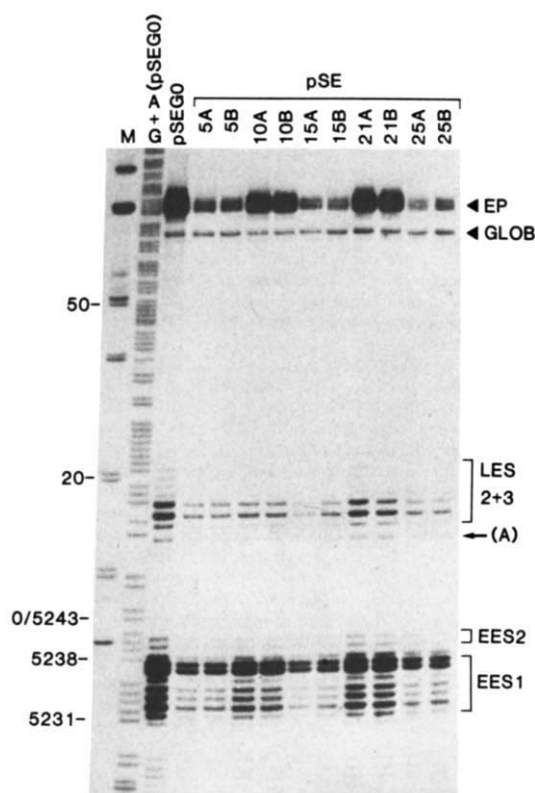


Fig. 2 Effect of insertions between the 21-bp repeat region and the enhancer on transcription initiated *in vivo* from the SV40 early-early and late-early start sites. HeLa cells were transfected with the pSE mutant series (as indicated at the top of each lane) and cytoplasmic RNA was analysed by quantitative S_1 nuclease mapping as described previously^{4,5}, using probes A and B which correspond to the [³²P]5'-end-labelled coding strand of the *Bst*NI fragment of the rabbit β -globin gene (position +138 to -84) and the [³²P]5'-end-labelled SV40 early coding strand of the *Hind*III to *Cla*I fragment of pSEGO, respectively. EP indicates the S_1 nuclease-resistant DNA fragment that arises from the homology between probe A and the SV40-globin hybrid transcripts. GLOB corresponds to the S_1 nuclease-resistant DNA fragment of probe A expected for RNA initiated at the start site of the reference β -globin gene contained in these recombinants. The S_1 nuclease-resistant bands EES1, EES2 and LES2+3 correspond to RNA initiated from the early-early and the late-early promoters, respectively, as described previously^{1,5} and in the text. The arrow labelled A points to a resistant band corresponding to start site A indicated in Fig. 1. Numbers on the left-hand side pointing to the A+G sequence ladder of probe B indicate the SV40 nucleotide positions using the BBB numbering system. Lane M, size markers ([³²P]5'-end-labelled *Msp*I digest of pBR322).

the same critical DNA turn-dependence as that observed with the 21-bp repeat region in its natural orientation. In mutant pSR0, initiation is low from EES1 and high from EES2 compared with the wild-type early promoter in pSO, and similar to that of mutants pS4 and pSB24 containing a 4-bp insertion between the 21-bp repeat region and the TATA box (Figs 3–5). This observation is particularly striking because the first G·C motif (VI) in pSR0 is at the same distance from the TATA box as the first G·C motif (I) in pS4 or pSB24, that is, approximately half a turn off from the natural configuration. Introducing deletions (pSR4, pSR5, pSR6 and pSR7) or an insertion of approximately one-half a DNA turn (pSR4) in pSR0, thereby rebuilding a wild-type architecture, resulted in a pattern of initiation from EES1 and EES2 that is very similar (EES1 much higher than EES2) to that observed with the naturally spaced promoter of pSO (Figs 1, 4, 5). Initiation from EES1 is the highest in the deletion mutant pSR6, whereas it is concomitantly the lowest from EES2 (Figs 1, 4). A drastic decrease in

RNA initiated at both EES1 and EES2 is observed with inserts of increasing length, but the pattern of initiation from these start sites shows the same dependence on DNA turn as in the pS series (compare pSR0, pSR10A and B and pSR21A and B with pSR4, pSR15A and B). We conclude that, even when inverted, the 21-bp repeat region has to be correctly aligned with the TATA box element to preferentially activate initiation of transcription from the EES1, and similarly will preferentially stimulate initiation from the EES2 when aligned with the T-rich substitute TATA box.

As in the case of the pS spacing mutant series, in which the 21-bp repeat region is in the normal orientation, initiation from the late-early start sites, LES2+3, of the pSR series increased with the length of the inserts. Finally, it should be stressed that all pSR recombinants contain a 4-bp insertion between the 21-bp repeat region and the enhancer. As introducing one-half a DNA turn between the enhancer and the 21-bp repeat region in its natural orientation results in a striking decrease of initiation from all start sites (pSE5 in Figs 2, 5), it is likely that these additional 4 bp are required to stereo-align some elements of the enhancer and the inverted 21-bp repeat region. In fact, the additional insertion of 5 bp between the 21-bp repeat region and the enhancer of pSR4 results in a strong decrease in initiation from all start sites (data not shown).

A role for protein–protein interactions

Our results are most easily interpreted along the lines that protein–protein interactions are involved in activation of transcription initiation. An alternative possibility, that factors bound to the 21-bp repeat region and the enhancer induce stereospecifically related local perturbations in the DNA helix that are recognized by the rest of the transcription machinery, appears less likely in the light of our present knowledge.

The results obtained by inserting odd and even multiples of half a DNA turn between the 21-bp repeat region and the normal or substitute TATA box regions demonstrate clearly that efficient activation of initiation of transcription requires a stereospecific alignment between these elements, and that the region at which initiation of transcription occurs is determined primarily by the position of the 21-bp repeat region. Studies *in vivo* (refs 3–5, 21 and refs therein) and *in vitro* (refs 2, 4, 22 and refs therein) have shown that the first three G·C motifs (most notably GC-I) of the 21-bp repeat region (Fig. 1) are essential elements of the SV40 early promoter. *In vitro*, the transcription factor Sp1 contacts several G residues (underlined) of the 3' GGCGGG 5' motif (refs 10, 11 and our unpublished results). A different factor binds to the TATA box (refs 7–9, 23; and our unpublished results). Thus, initiation of transcription appears to require a stereospecific alignment between the Sp1 and TATA box factors, which led us to propose the following model.

The transcription machinery (presumably RNA polymerase B) interacts primarily with at least the first of the three Sp1 factor molecules (bound to G·C motifs I–III) which are all located on one side of the DNA helix. The TATA box factor, when correctly positioned by a properly aligned TATA box, interacts with the polymerase and directs it to initiate principally at the corresponding start site(s) (EES1 in the wild type; mutating the TATA box results in the disappearance of RNA initiated at the EES1 both *in vivo* and *in vitro*¹). Misalignment caused by inserting half a DNA turn leads to the preferential use of the T-rich substitute TATA box which is located on the other side of the helix and controls initiation at the EES2. Sp1 factors bound to G·C motifs II and III may either interact directly with the transcription machinery or interact with each other and their effect be mediated by the Sp1 factor bound to G·C-I. Whether this Sp1 factor and the TATA box factor interact directly with each other as well as with RNA polymerase, for instance to stabilize their respective interactions with their cognate binding sites, cannot be predicted from our present study. However, stable binding of Sp1 factor to the 21-bp repeat region *in vitro*

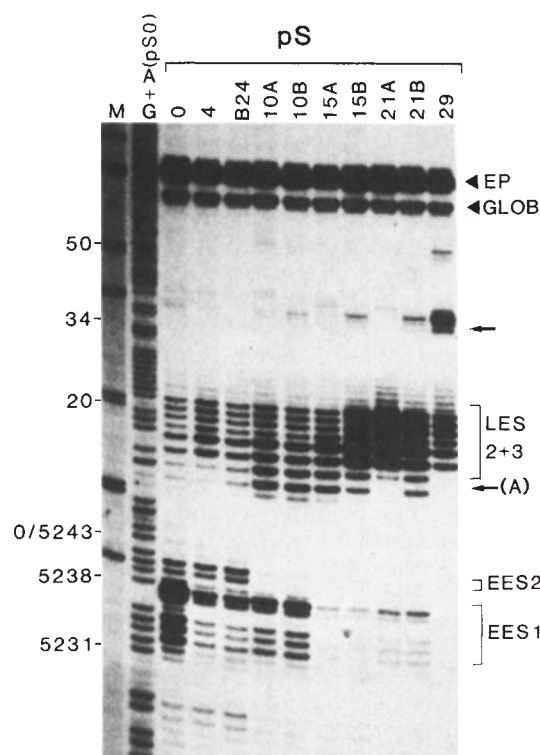


Fig. 3 Effect of insertions between the 21-bp repeat and the TATA box regions on transcription initiated *in vivo* from the SV40 early-early and late-early start sites. HeLa cells were transfected with the pS mutant series (as indicated) and cytoplasmic RNA was analysed by quantitative S_1 mapping using a probe B prepared from pSO. Symbols are as described in Fig. 2 legend. The arrow on the right-hand side indicates the beginning of all insertions (SV40 coordinate 34). Start site A corresponds to position 11.

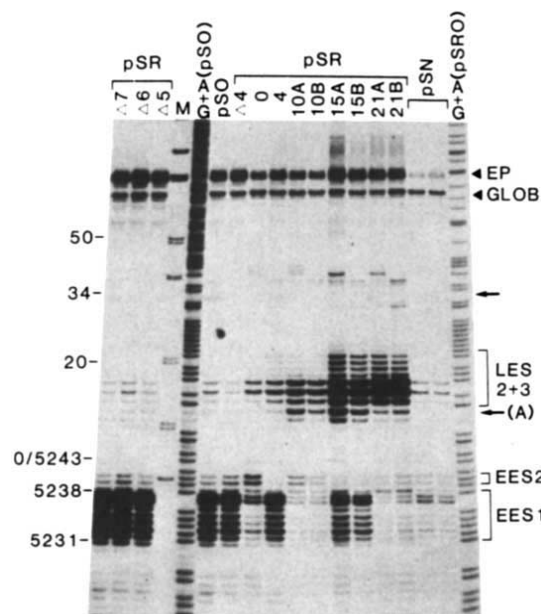


Fig. 4 Effect of deletion and insertion between the inverted 21-bp repeat region and the TATA box region on transcription *in vivo* from SV40 early-early and late-early start sites. Cytoplasmic RNA extracted from HeLa cells transfected with the pSR series mutants (as indicated) was analysed by quantitative S_1 nuclease mapping using a probe B prepared from pSO. Symbols are as described in the legends to Figs 2, 3. The arrow on the right-hand side of the figure indicates the beginning of all insertions (position 34). Start site A corresponds to position 11.

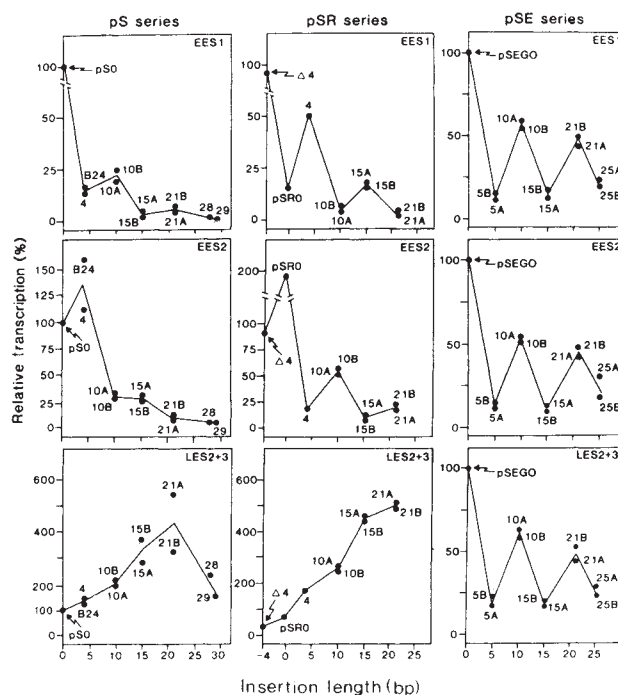


Fig. 5 Diagrammatic comparison of the results obtained with the various insertion mutants. The length of the insertions (or deletion, pSR series) between the 21-bp repeat region and the TATA box region or the enhancer is indicated on the abscissa and the relative efficiency of transcription is given on the ordinate. The left-hand panels represent the results of the pS mutant series for transcription starting at EES1, EES2 and LES2+3, whereas the right-hand and centre panels show the results for the pSE (insertions between the TATA box and the 21-bp repeat region) and pSR (insertions between the TATA box and inverted 21-bp repeat region) mutant series, respectively. The values which are taken from Fig. 1 are expressed relative to EES1, EES2 and LES2+3 of pSO (for the pS and pSR series) and pSEGO (for the pSE series).

does not require the presence of the TATA box region²³. Increasing the distance between the 21-bp repeat region and the TATA box (or its substitute) either prevents the transcription machinery from interacting efficiently with the bound TATA box factor (even when properly aligned) or prevents the TATA box factor from binding efficiently to the TATA box, and results in the observed rapid decrease in initiation from the EES1 and EES2. Weak substitute TATA box elements will then direct the transcription machinery to preferentially initiate at the various LES. With longer inserts, similar weak multiple substitute TATA box elements (see ref. 24) will direct the transcription machinery to initiate at multiple sites in the 30–50-bp region located immediately downstream from the 21-bp repeat region. Alternatively, RNA polymerase bound to the 21-bp repeat region may initiate at multiple sites without the help of the TATA box factor. Such a model readily accounts for the observation that there is no marked overall decrease in RNA initiated from the SV40 early promoter region in the insertion mutants (see band EP in Fig. 3, whose intensity reflects the total amount of RNA initiated from the SV40 early promoter region).

The study of the pSR series shows clearly that the 21-bp repeat region, even when inverted, has to be stereo-aligned with the TATA box or the substitute T-rich TATA box to preferentially stimulate initiation at the EES1 or EES2, respectively. It is particularly striking that the pattern of initiation of the wild-type naturally spaced pSO recombinant (EES1 much higher than EES2) is best reproduced with the inverted 21-bp repeat region recombinants pSR4 and pSRΔ6, whose position of the corresponding G-C-rich motifs is separated by 10 bp when their

TATA boxes are aligned. Thus, our results suggest strongly that equivalent protein-protein contacts can be established between the transcription machinery and the factor(s) bound to the G-C motifs, irrespective of the orientation of the 21-bp repeat region. This is very surprising because there is no apparent symmetry in the sequence of these motifs. Several explanations can be proposed to account for this paradox: (1) symmetry may be present in the G-C motif, even though it is not readily apparent; (2) Sp1 factor could be an asymmetrical molecule with equivalent protein interaction sites at either end; (3) the protein with which Sp1 factor interacts (for instance, RNA polymerase which has multiple subunits) may possess symmetrical protein interaction sites; (4) Sp1 factor may be composed of two functional domains linked by a flexible stem: one DNA-binding domain which would specifically recognize the G-C motifs, and one protein-interacting domain which would interact with some component of the transcription machinery. We find the two-domain hypothesis attractive because such separate domains for DNA binding and activation of transcription by protein interaction with RNA polymerase are known to exist in repressor proteins of *Escherichia coli* λ phages²⁵⁻²⁷. In this respect, we note that in the two inverted 21-bp region recombinants pSR4 and pSRΔ6 (see above) and in the wild-type recombinant pSO (which exhibit the same patterns of initiation at the EES1 and EES2), the N-7 of the G residues in the G-C motifs, which are protected by Sp1 factor from methylation by dimethylsulphate¹¹, are located on the same side of the DNA helix when their TATA box sequences are aligned (data not shown).

Our present results and those reported elsewhere⁶ demonstrate that efficient initiation of transcription from the entire SV40 early promoter region requires a stereospecific alignment between some element(s) contained in domain A of the enhancer and the 21-bp repeat region. Transcriptional factors are known to bind *in vitro* to domain A^{13,14,16}. Thus, it is likely that they have to be stereo-aligned with Sp1 factors for the enhancer to efficiently stimulate transcription. Note that the Sph motifs present in domain A, known to be important for enhancer activity⁶, are separated by approximately one helical turn from one another and by a multiple of 10 bp from the G-C motifs. Thus, protein(s) bound to these Sph motifs and to the G-C motifs may be on the same side of the helix, and may specifically interact with each other and/or independently with some element(s) of the transcription machinery (for example, RNA polymerase; see above). Note that Sp1 factor can bind to the 21-bp repeat region *in vitro* in the absence of the enhancer sequence^{10,23}, whereas proteins appear to be less stably bound to the enhancer in the absence of the 21-bp repeat region¹⁶, and that transcription can occur *in vitro* (but not *in vivo*) from an enhancer-less early promoter, although at a reduced rate^{2,14}.

It appears, therefore, that the assembly of proteins bound to the various elements of the SV40 early promoter could constitute a broad protein interaction region located mainly on one side of the DNA helix, where it could act as a specific recognition region for RNA polymerase B, which itself is composed of

multiple subunits²⁸. The part played by the transcriptional factors bound to the 21-bp repeat and the TATA box region would be to anchor stably RNA polymerase to enable it to initiate at the start sites, and perhaps also to have a role in its activation. The protein(s) bound to enhancer domain A would participate in activation of transcription initiation, either directly by interacting with RNA polymerase, or indirectly through contacts with the protein(s) bound to the 21-bp repeat region. All these interactions contribute to increase the transcription machinery concentration at a given promoter region which has to compete successfully with the rest of the very large genomic DNA for efficient initiation of transcription to occur. A DNA looping mechanism may account for the persistence of some enhancer activity (see refs 1, 19) as it is moved away from the other promoter elements. That the enhancer, and not only the 21-bp repeat region, can activate transcription irrespective of its orientation, can be explained by the hypothesis that proteins bound to it are composed of two distinct functional domains linked by a flexible stem (see above). The fact that enhancer domain B (essential for enhancer activity) apparently does not need to be stereospecifically aligned makes it unlikely that proteins bound to that domain belong to the broad RNA polymerase B binding and activation complex discussed above. However, *in vitro* studies^{13,14} indicate that proteins bound to domain B are involved in the stimulation of transcription observed *in vitro* with templates that contain enhancers, suggesting that, nevertheless, these proteins interact in some way with the transcription machinery.

In conclusion, our results clearly support the view that multiple protein-protein interactions have a key role in the generation of an active transcriptional initiation complex at a promoter, which is a very large specific nucleoprotein structure composed of multiple sequence elements recognized by different cooperating factors. It is likely that this concept can be generalized to other class B(II) promoters, as the same DNA turn dependence has been observed when the SV40 enhancer activates transcription from the adenovirus-2 major late promoter (our unpublished results). *In vitro* studies aimed at reconstructing transcription initiation complexes from purified factors and RNA polymerase are in progress to investigate the validity of our model.

We thank J. Wang and M. Lewitt for helpful discussions; R. Breathnach, B. Wasylyk, G. Albrecht and I. Davidson for critical reading of the manuscript; our colleagues for generous gifts of recombinants; M. Acker for growing cells; A. Staub for synthesizing oligonucleotides; C. Werlé and B. Boulay for illustrations; and the secretarial staff for typing the manuscript. This work was supported by CNRS (ATP 3582), INSERM (PRC 124026), the Fondation pour la Recherche Médicale, the Ministère de la Recherche et de la Technologie (82V1283) and the Association pour le Développement de la Recherche sur le Cancer. K.T., A.W. and M.Z. were recipients of fellowships from INSERM/JSPS and CNRS, Natural Sciences and Engineering Research Council of Canada and the CNRS/Deutsche Forschungsgemeinschaft, respectively.

Received 3 September; accepted 15 November 1985.

- Wasylyk, B., Wasylyk, C., Matthes, H., Wintzerith, M. & Chambon, P. *EMBO J.* **2**, 1605-1611 (1983).
- Vigneron, M., Barrera-Saldana, H. A., Baty, D., Everett, R. E. & Chambon, P. *EMBO J.* **3**, 2373-2382 (1984).
- Buchman, A. R., Fromm, M. & Berg, P. *Molec. cell. Biol.* **4**, 1900-1914 (1984).
- Barrera-Saldana, H. A. *et al.* *EMBO J.* (in the press).
- Baty, D., Barrera-Saldana, H. A., Everett, R. D., Vigneron, M. & Chambon, P. *Nucleic Acids Res.* **12**, 915-932 (1984).
- Zenke, M. *et al.* *EMBO J.* (in the press).
- Davison, B. L., Egly, J. M., Mulvihill, E. R. & Chambon, P. *Nature* **301**, 680-686 (1983).
- Parker, C. S. & Topol, J. *Cell* **37**, 273-283 (1984).
- Dynan, W. S. & Tjian, R. *Cell* **32**, 669-680 (1983).
- Dynan, W. S. & Tjian, R. *Cell* **35**, 79-87 (1983).
- Gidoni, D., Dynan, W. S. & Tjian, R. *Nature* **312**, 409-413 (1984).
- Sassone-Corsi, P., Dougherty, J. P., Wasylyk, B. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* **81**, 308-312 (1984).
- Sassone-Corsi, P., Wildeman, A. G. & Chambon, P. *Nature* **313**, 458-463 (1985).
- Wildeman, A. G., Sassone-Corsi, P., Grundström, T., Zenke, M. & Chambon, P. *EMBO J.* **3**, 3129-3133 (1984).

- Sergeant, A., Bohmann, D., Zentgraf, H., Weiher, H. & Keller, W. *J. molec. Biol.* **180**, 577-600 (1984).
- Wildeman, A. G. *et al.* *Molec. cell. Biol.* (submitted).
- Moreau, P. *et al.* *Nucleic Acids Res.* **9**, 6047-6068 (1981).
- Banerji, J., Rusconi, S. & Schaffner, W. *Cell* **27**, 299-308 (1981).
- Wasylyk, B., Wasylyk, C. & Chambon, P. *Nucleic Acids Res.* **12**, 5589-5608 (1984).
- Wang, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 200-203 (1979).
- Everett, R. D., Baty, D. & Chambon, P. *Nucleic Acids Res.* **11**, 2447-2464 (1983).
- Hansen, U. & Sharp, P. A. *EMBO J.* **2**, 2293-2303 (1983).
- Miyamoto, N. G., Moncollin, V., Egly, J. M. & Chambon, P. *Nucleic Acids Res.* **12**, 8779-8799 (1984).
- Wasylyk, B., Wasylyk, C., Augereau, P. & Chambon, P. *Cell* **32**, 503-514 (1983).
- Hochschild, A., Irwin, N. & Ptashne, M. *Cell* **32**, 319-325 (1983).
- Wharton, R. P., Brown, E. L. & Ptashne, M. *Cell* **38**, 361-369 (1984).
- Ptashne, M. *Trends biochem. Sci.* **9**, 142-145 (1984).
- Kedinger, C., Gissinger, F. & Chambon, P. *Eur. J. Biochem.* **44**, 421-436 (1974).
- Toozé, J. (ed.) *DNA Tumor Viruses* (Cold Spring Harbor Laboratory, New York, 1982).
- Matthes, H. W. D. *et al.* *EMBO J.* **3**, 801-805 (1984).
- Grundström, T. *et al.* *Nucleic Acids Res.* **13**, 3305-3316 (1985).
- Wallace, R. B. *et al.* *Gene* **16**, 21-38 (1981).

A very high energy γ -ray burst from the Crab pulsar

P. N. Bhat, P. V. Ramanamurthy, B. V. Sreekantan
& P. R. Vishwanath

Tata Institute of Fundamental Research, Homi Bhabha Marg,
Bombay 400005, India

The Crab pulsar, a source of pulsed photons over a wide range of energies, has been observed in the TeV energy range by several groups using the Atmospheric Cerenkov technique¹. Time-averaged phasograms have shown signals on only a few occasions. The Durham University group² detected pulsed TeV γ -rays during two transients lasting 15 min, each in October, 1981, while the Ooty group^{3,4} has shown that the transient duration could be on time scales of minutes or even seconds. In our recent observations, we identified a 15-min interval on 23 January 1985 during which pulsed TeV γ -rays were detected at a 5.1 sigma level at the same phase as the radio main pulse. Our inference of transient pulsed emission is strengthened by the absence of a signal in a nearby control telescope tilted 8° away from the pulsar and evidence for signal in another telescope 11 km away. We present here an analysis of the data from our 1984–1985 observations.

The Ooty array (for details, see refs 3 and 5), normally operated with 18 mirrors, was split up and operated at two sites, site A and site B, 11 km apart. At site A, eight large mirrors, with a diameter of 1.5 m, were used and at site B, 10 small mirrors, with a diameter 0.9 m were used. For some of the runs at site A, the eight large mirror array was further split into two halves with one half of the array (A1) pointing at Crab and the other half (A2) at a point 8° south of Crab. The trigger was derived from a three-out-of-four majority logic at site A and a coincidence of four mirror banks (at least two mirrors in each bank) at site B. The chance coincidence rates of the arrays were also monitored. The arrival time of the event was recorded to an accuracy of 0.1 ms at both the sites. In addition, the pulse from the photomultiplier on each mirror at site A was digitized with a LeCroy ADC (analog digital converter). The time keeping accuracy was 0.03 ms which was achieved with a NNSS (Navy Navigation Satellite System) satellite timing receiver. The average trigger rate at site A was 500 per min when the array was not split and about 300 per min for each of the half arrays (A1 and A2) when it was split. The average rate at site B was about 120 per min. Such high rates resulted from several factors: large mirror areas, majority logic and 4° apertures employed in the experiment. These rates convert to a threshold of 800 GeV for the full array at site A and 1.2 TeV for arrays A1 and A2. The energy threshold at site B was 2.2 TeV.

Data were taken during December 1984 and January and February 1985. The February runs were rejected because of persistent lightning. Data on Crab pulsar were taken on nine nights at site A, out of which the two array (A1 and A2) mode was used on four nights. At site B, there were 11 nights of observation, out of which there was overlap with observations at site A during six nights only. Total observation times at sites A and B were 1,436 and 1,908 min respectively. The total time of simultaneous observations at the two sites was 1,004 min, out of which the array at site A was operated in the two half array mode for 540 min.

The analysis of the data was carried out using the pulsar elements applicable to the epoch of our observations obtained from contemporaneous radio observations (by A. G. Lyne). A barycentric time reduction programme and related ephemeris (due to D. Richards and I. Shapiro respectively) were used to compute the number of Crab pulsar cycles at the barycentre for every half hour at the observation site. The pulsar phase for each event was then computed by interpolation (checked to be

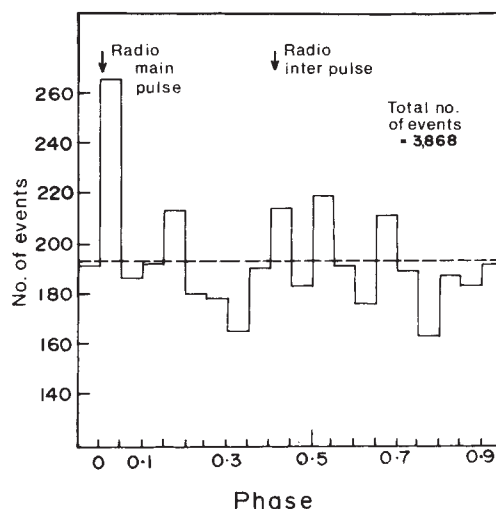


Fig. 1 The phasogram of events from array A1 looking at the Crab pulsar during the 15-min duration 1711 to 1726 h on 23 January 1985. Each bin has a width of 1.65 ms. The average for the whole phasogram is shown by the horizontal dashed line.

accurate to better than 0.0016%) and folded to obtain the phasogram for the pulsar. The number of bins used for the phasogram is 20.

The data were split into mini-runs of duration five minutes each and a phasogram was constructed for each mini-run. Phasograms from three consecutive mini-runs were added to get one 15-min phasogram. There were such 116 independent 15-min phasograms from data at site B and 90 from site A, giving a total of 206 phasograms. When these phasograms were inspected for significant peaks, we found one 15-min interval during the night of 23 January 1985 from the half array A1 (looking at the Crab pulsar) at site A showing a 5.1 sigma peak at the position of the radio main pulse. In the phasogram, which is shown in Fig. 1, there are 72 excess events during the 15-min interval in the first phase bin when the average was 193 events. There is a minor (1.5 σ) peak at the position of the radio inter-pulse. Figure 2a, b shows the observed number of events in the first phase bin and the average per bin from the entire phasogram for all the three mini-runs from arrays A1 and A2 looking at and away from the pulsar respectively. While A1 has shown excess of $>2\sigma$ in all the three mini-runs, there is a non-significant excess only in the first mini-run in A2. The total number of excess events in the first phase bin from data at A2 is only seven when the average was 182 events per phase bin.

The array at site B had a higher energy threshold and since the photomultiplier pulses at site A were digitized, one can check whether the response at site B is commensurate with the energy spectrum of events at site A. From matching the ADC response with the number of events from site B, one can pick out the higher energy events. Figs 2c and 2d show the behaviour of higher energy events in the array A1 at site A and of all events at site B. In the first two mini-runs, the response of the two arrays is quite similar: 79 events were observed in the first phase bin at each of the two arrays when the average number of events per phase bin is 64. However, the array at site B seems to be deficient in the third mini-run. Therefore, we can only say that the two arrays have behaved similarly for at least 2/3 of the duration. We note that the trigger rates and chance coincidence rates at the two arrays did not show any significant increase during these 15 min.

The Poissonian probability that an excess of 72 or more events is seen when the average is 193, amounts to 6.1×10^{-7} . However, there were 206 independent 15-min intervals and each one was made up of three mini-runs of five minutes. Therefore, the probability has to be multiplied by 618, which is the total number of ways in which the 15 min intervals could be formed. Thus,

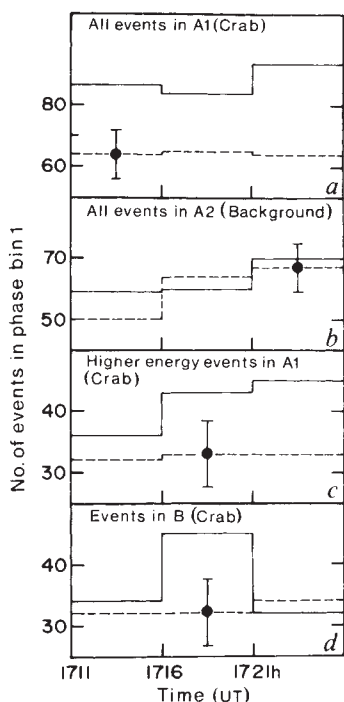


Fig. 2 Solid lines are the observed number of events in the phase bin 1 (0. to 0.05 in phase) during a 15 min interval 1711 to 1726 h (UT) on 23 January 1985 and the broken lines are the average per bin based on the full phasogram. Typical 1σ error bars are shown in each case. *a* Events in the array looking at Crab at site A. *b* Events in the array looking at a point 8° south of Crab. *c* Higher energy events in the array looking at Crab at site A—the cut was made on energy such that the event rate matches with the one at site B. *d* All events in the array looking at Crab at site B.

the Poissonian probability that the peak is at the position of the radio main pulse due to chance turns out to be 3.8×10^{-4} . A more, conservative estimate for the same is obtained by O'Mongain's likelihood analysis⁶ which is 4.2×10^{-3} . At this juncture, one has to ask about the similarity of VHE γ -ray phenomena with those seen by the COS-B (7) group at lower energies from the Crab pulsar. As some of the analyses of the VHE γ -ray observations did not have absolute phase information, it is not possible to say at what phase the signal was seen whenever a claim was made. In some cases, absolute phase information was available (for example, see ref. 1), but the phase of the VHE gamma ray signal did not coincide with that of the signal at lower energies. Therefore, if we accept that there could be phase differences between γ -ray emissions at different energies, one has to multiply the above probabilities by the number of bins which is 20. Therefore, the probability that the signal could appear in any bin due to chance is 7.6×10^{-3} (Poissonian). Thus, the confidence level for a VHE γ -ray signal in our data is 92% (O'Mongain) and 99.2% (Poissonian).

We have tried to verify the authenticity of the signal seen by us by changing the true Crab pulsar period in steps of $\Delta P = 10^{-7}$ s and computing the phasograms for each period. The step size of ΔP is chosen such that the neighbouring trials are independent of each other. 10,000 different periods were tried with 5,000 on either side of the true period; there was not even one trial in which a signal $>5\sigma$ was seen. The probability of seeing a $>5\sigma$ signal in any phase bin is therefore $<6.9/10,000 = 6.9 \times 10^{-4}$ with 99.9% confidence level which is much higher than the value of 92% derived from the likelihood analysis of O'Mongain. The latter may therefore be considered very conservative.

As noted earlier, two such 15-min bursts were seen by Gibson *et al.* in October 1981. The similarities between those and the present burst are that the duration is 15 min and the excess number of events is 70. Whereas the Durham observations did

not have absolute phase information and the pulse had a long duration of 6 ms FWHM (full width at half maximum), the peak seen by us coincides with the radio main pulse. The pulse width of the peak in our data is <2 ms consistent with lower energy signals⁷. Finally, Gibson *et al.* saw a $18 \pm 7\%$ increase in the trigger rate during a 15-min interval on 23 October 1981. The increase in trigger rate of our array A1 was $5 \pm 4\%$, consistent with no increase. This particular burst seen by us was potentially observable only in those experiments operating from sites in the longitude range 30° E to 105° E during the time 1711 h to 1726 h on 23 January 1985. The time-averaged flux of γ -rays in the burst at site A over its duration of 15 min is calculated to be $(2.5 \pm 0.6) \times 10^{-10}$ γ per $\text{cm}^2 \text{ s}^{-1}$ at $E_\gamma > 1.2$ TeV. The Durham group⁷ reported a flux of $(2.0 \pm 0.3) \times 10^{-10}$ γ per $\text{cm}^2 \text{ s}^{-1}$ at $E_\gamma > 3$ TeV. They considered 34% of their events to be periodic. It appears that the phenomenon of transient pulsed emission occurs only very rarely. Neither Dowthwaite *et al.*⁸ nor ourselves saw any more occurrences in observation times of 100 h each. There may also be a persistent weak pulsed emission from the Crab pulsar at the radio main phase⁸.

Our conclusion that we are seeing a genuine pulsed emission from the Crab pulsar lasting for 15 min during the night of 23 January 1985 is strongly supported by the presence of a 5.1σ signal in one of the phase bins of the data from an array looking at the Crab pulsar; the absence of any signal in the data from an array looking simultaneously at background; the simultaneous presence of a signal in the same phase bin from an array situated 11 km away and looking at the pulsar; and the failure to obtain a 5σ peak in any phase bin when the same data were analysed in an identical fashion using 10,000 wrong periods. The fact that the signal is seen at the radio main phase shows that at least some of the VHE γ -ray phenomena stem from the same hot spot governing the lower energy processes.

We thank A. R. Apte, N. V. Gopalakrishnan, R. Mahalingam, S. Swaminathan and M. Venkateshwarlu for help in running the experiment; S. C. Tonwar for assistance with data at site B and A. G. Lyne for supplying pulsar elements.

Received 2 August; accepted 24 October 1985.

- Porter, N. A. & Weekes, T. C. *Smithson. astrophys. Obs. spec. Rep.* 381 (1978).
- Gibson, A. I. *et al. Nature* **296**, 833–835 (1982).
- Vishwanath, P. R. *Proc. International Workshop on Very High Energy Gamma Ray Astronomy* (Ootacamund, India), (eds P. V. Ramanamurthy & T. C. Weekes) 21–42 (1982).
- Bhat, P. N. *et al. Adv. Space Res.* **3**, 135–138 (1982).
- Ramanamurthy, P. V. *Non Solar Gamma Rays*, 71–84 (Oxford, Pergamon, 1979).
- O'Mongain, E. *Nature* **241**, 376–379 (1973).
- Wills, R. D. *et al. Nature* **296**, 723–746 (1982).
- Dowthwaite, J. C. *et al. Astrophys. J.* **286**, L35–L38 (1984).

Solar ellipticity fluctuations yield no evidence of g-modes

J. R. Kuhn*, K. G. Libbrecht† & R. H. Dicke*

* Joseph Henry Laboratories, Princeton University, Princeton, New Jersey 08544, USA

† Solar Astronomy 264–33, California Institute of Technology, Pasadena, California 91125, USA

Although there have been several claims for the detection of solar g-modes with periods between 2 and 10 hours^{1–3} and although the present sensitivity of the Princeton Solar Distortion Telescope should allow these low frequency modes to be observed, solar oblateness data from the summers of 1983 and 1984 show no evidence of such oscillations with periods between 1 and 5 hours. In about 250 days (nearly 1,000 hours) of observations, we find no evidence for significant spectral power associated with g-modes. In particular, there is no evidence of a 160.01-min period solar oscillation.

The surface velocity amplitudes of solar g-modes appear to be between 0.1 and 1 m s^{-1} , but there is little agreement between

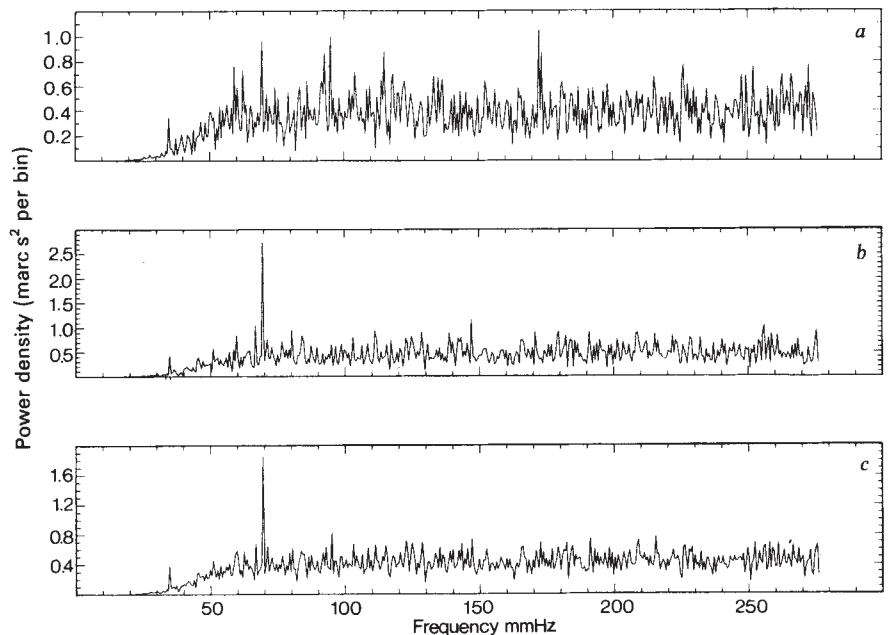


Fig. 1 *a*, Average of the least-squares spectra from the six 1983 data sets. *b*, Same as *a* but for 1984 datasets. *c*, All 12 spectra averaged from 1983 and 1984.

different observers on the excitation spectrum. Several groups have, however, claimed detection of a 160.01-min period solar oscillation^{4,5} also with a velocity amplitude that seems to vary between 0.17 and near 1 m s⁻¹. All of these modes have been observed with low spatial resolution and are most probably of low spherical harmonic degree ($l \leq 2$).

It is notable that the surface amplitudes of these oscillations are comparable to 5-min *p*-mode amplitudes of about 15 cm s⁻¹ (ref. 6). If the observations are correct, the energy per *g*-mode is roughly 10⁸ times larger than typical *p*-mode energies, and is thus of considerable interest.

Except for the ACRIM data³ and Hill's data⁷ most of the low frequency data are due to Doppler velocity observations. Since the period of the modes discussed in this paper are long compared to a radiative relaxation time at the photosphere ($\sim 10^3$ s) the pressure density perturbations due to the oscillation will be nearly isothermal. It is therefore reasonable to calculate the shape change by following photospheric brightness contours as they move due to mass motion of the oscillation displacement field below the photosphere.

The distortion telescope and oblateness data have been described in detail elsewhere⁸⁻¹⁰. In brief, measurements of the deviation from a circular solar limb shape are obtained by measuring the flux from a solar image occulted by a slightly undersize circular occulting disk. The solar flux is measured in two broadband colours (0.5 μ m and 0.8 μ m) and at 256 positions around the limb. Between 9 and 20 arc seconds of the limb extends beyond the occulting disk. The data are obtained with a specialized telescope now operating at the Mount Wilson Observatory.

A data set at one of three possible limb exposures and two simultaneous colours is obtained approximately once every 5 min. The flux measurements in 128 distinct position angles (only symmetric distortions about the image centre may be observed) around the image are reduced to a displacement in arcseconds using a geometrical calibration. Corrections for optical imperfections of the telescope, mirror distortions, atmospheric transparency and refraction and facular contamination are applied to the data before obtaining the two coefficients that describe the oblateness of the solar image. For the discussion below the oblateness is described by the coefficients *D* and *V* of a fit of $D \sin 2\theta + V \cos 2\theta$ to the binned shape data. Here θ measures the angle from the solar rotation axis projected onto the image plane (and the *V* and *D* coefficients determine the shape and orientation of the solar oblateness in a frame fixed with respect

to the telescope). Thus each observation at a given limb exposure yields four coefficients—a *V* and *D* term for each of the two colour bands. Except for additional atmospheric corrections described by Dicke *et al.*¹¹ the analysis sketched above essentially follows the description in the references.

The two summers of observations yielded about 254 useful days of data, amounting to about 11,000 5-min observations distributed between the three possible limb exposures. After subtracting least-squares fitted daily quadratic polynomials, to remove daily trends, the standard deviation of the residuals was approximately 24 milliarcseconds in *D* or *V*. The oblateness data will not be sensitive to $l=0$ or 1 oscillations, but *g*-modes with $l=2, 3, \dots$ will contribute to the observed power in the *V* or *D* coefficients. If the 160 min mode is, for example, an $l=2$ *g*-mode, it will also contribute to the shape data.

For definiteness we will estimate the expected shape signal amplitude from the assumption that the 160 min mode is an $n=9, l=2$ *g*-mode (the closest predicted frequency for low order modes). The velocity amplitude of an $m=0$ mode can be written

$$\tilde{V}_n(r, \theta) = V_n(r) P_2(\cos \theta) \hat{r} + \frac{U_t(r)}{r} \frac{\partial P_2(\cos \theta)}{\partial \theta} \hat{\theta} \quad (1)$$

A model calculation¹² gives for the $n=9$ mode $V_9(R_{\text{sun}})/U_9(R_{\text{sun}}) = 1.14$. The line-of-sight velocity V_{0n} can be related to the modal amplitude V_n from

$$V_{0n} = S_n \cdot V_n \quad (2)$$

where V_{0n} is the intensity weighted line-of-sight velocity, $\tilde{V}_n \cdot \hat{z}$, integrated over the solar disk. The factor S_n is easily evaluated for full disk observations and has been tabulated¹³. For example, $S_9 = 1.3$ for the radial-transverse velocity ratio given above for the $n=9, l=2$ mode. Other long period $l=2$ modes have values of S_n within about 50% of this value. Projecting the $P_2(\cos \theta)$ term against $\sin 2\theta$ or $\cos 2\theta$ gives an approximate relation between the coefficient *V* and the observed full disk velocity amplitude, V_0 of a *g*-mode of frequency ω ,

$$V \approx 1.3 V_0 / \omega \quad (3)$$

Thus, from the 160 min mode with an observed amplitude of about 1 m s⁻¹ we expect an oblateness coefficient of $V \sim 2 \times 10^3 m = 2.7$ milliarcsec. If the oblateness residuals were uncorrelated such an oscillating shape signal would be observable with high signal-to-noise.

Below we address two questions: is there evidence of a 160.01-

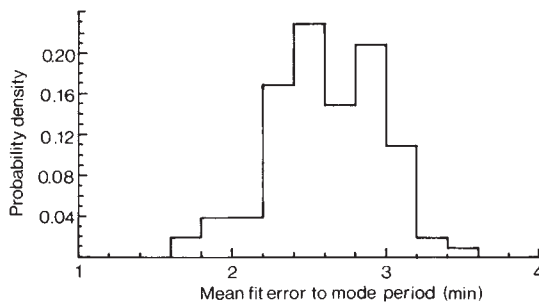


Fig. 2 Distribution of the mean fit errors to the g-mode asymptotic period relation from 100 synthetic peak lists.

min solar oscillation in these data? and is there evidence of any other solar oscillating power in the oblateness residuals? These questions are complicated by the very uneven sampling in time of the data. For example, in 1984 there were about 5,500 observations between day number 107 and 274, spaced at about 5-min intervals. Any mode structure will be aliased in the observed power estimates.

Data from the two colour channels are obtained simultaneously and are highly correlated. Thus to reduce the noise the colours are averaged before computing power spectra. By averaging the power spectrum from three possible limb exposures and both V and D coefficients we may hope to find a 160-min signal. This has been done in Fig. 1 for 1983 and 1984 data separately, and combined. 'Power spectra' are computed from the summed squared amplitude of least-squares fits of $\sin \omega t$ and $\cos \omega t$ to the colour averaged residuals.

Figure 1c is an average of 12 'pseudo-spectra' and is our first approximation to the actual mean power spectrum. We note that at frequencies near $104.16 \mu\text{Hz}$ ($T = 160.01$ min) all peaks are smaller than twice the mean power level. To the extent that this approximates the actual mean spectrum the probability distributions of signal in the presence of noise calculated by Groth¹⁴ are applicable here. We find that there is less than a 1% chance that the signal power at $f = 104.16 \mu\text{Hz}$ is larger than the mean noise power of about 0.4 milliarc^2 . It is notable that there are prominent peaks in the averaged and yearly spectra at periods of $1/10$, $1/9$, $1/6$, $1/5$ and $1/3$ days. Some 1 per day frequency sidelobes of the stronger peaks are also prominent.

Since we know the frequency and phase of the 160.01-min signal we can also fit it directly in the data. To simplify the analysis the data were again averaged by colour and limb exposure and binned in half-hour intervals to obtain a single

time series for each V and D coefficient for each year. The phase and period described by Grec *et al.*¹⁵ have been used to find the 160-min amplitude and fit error for 1983, 1984 and combined datasets, displayed in Table 1. The results of a non-linear fit with frequency treated as an adjustable parameter are also displayed.

Only the V coefficient of the 1984 data shows a statistically significant amplitude at about the 2σ level, but even this is probably due to the nearby strong peak at a period of 159.90 min. The results in Table 1 provide no evidence for the 160.01-min oscillation with an amplitude greater than, approximately, the fit uncertainty of 0.4 milliarcsec.

Several approaches have been considered to look for other discrete frequency power contributions to the data. (The first technique we examined was used originally by Scherrer¹ for finding g-modes, and as we will show below is not effective.)

Scherrer¹ claimed detection of g-modes by searching for peaks in the Stanford data using an iterative least-squares fitting technique (the 'clean' algorithm) and then noted that the peaks fit the asymptotic expression for the mode periods, $T(n, l)$:

$$T = T_0(n + l/2 - 1/4)\sqrt{l(l+1)} \quad (4)$$

where n and l are chosen separately for each mode to best fit the observed periods using a single value of T_0 . With 14 power spectrum peaks with periods between 172 and 329 min Scherrer found a good fit with $T_0 = 38.6$ min and l and n values between 1 and 2, and 6 and 20. As a test of the uniqueness of such a fit we have simulated this procedure on 100 sets of random numbers evenly distributed in frequency between the corresponding limiting periods in Scherrer's data. Each set of 14 'peaks' is used to estimate a value of T_0 which minimizes the summed squared deviation of the period calculated from equation (4) and random peaks. The integer n and l values are constrained to be less than or equal to 25 and 2 respectively and are adjusted to minimize the error for each trial T_0 value. A 1% resolution search between 30 and 50 minutes finds a value of T_0 which minimizes the summed error. The 100 trials yielded the mean error distributions shown in Fig. 2. Notice that such a fit is not very conclusive since an r.m.s. error of about 3 min is often possible given the range of available parameters. From Scherrer's n , l and T_0 values we calculated an r.m.s. value of 3.0 min from his data. Thus his fit for T_0 provides no supporting evidence that the power spectrum peaks are of solar origin.

The second approach we tried for finding discrete power contributions was to reduce the aliased structure in the spectrum by deconvolving the window function from the observed power spectra. For this purpose the data were averaged over the three

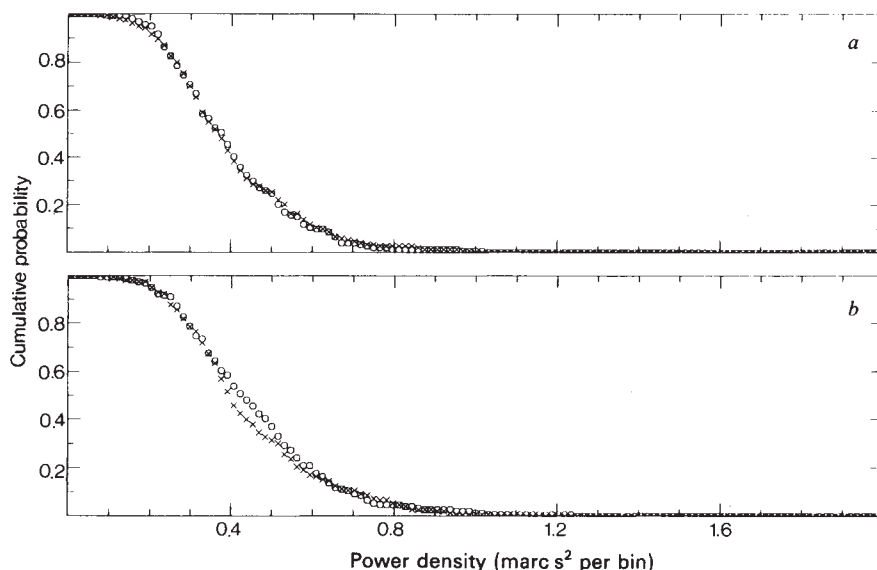


Fig. 3 a, Cumulative power distribution of the data in Fig. 1a (crosses) and the spectra of normally distributed white noise (circles). b, Same as a, except for 1984 data and time domain.

Table 1 Least-squares fit for 160.01-min oscillation

		1983	1984	Combined
$f = 104.16 \mu\text{Hz}$				
amplitude	V	0.4 ± 0.5	1.7 ± 0.8	0.3 ± 0.4
(marcs)	D	0.3 ± 0.5	0.6 ± 0.8	0.3 ± 0.4
Frequency (μHz)	V	103.92 ± 0.02	104.23 ± 0.03	104.235 ± 0.003
	D	104.18 ± 0.01	104.00 ± 0.05	104.171 ± 0.003
Amplitude	V	1.3 ± 1.3	2.1 ± 2.0	1.7 ± 0.8
	D	1.8 ± 1.3	1.3 ± 1.9	1.4 ± 0.8

limb exposures and two colours and binned in half-hour intervals. Under these conditions the 'filling factor' on the evenly spaced half-hour domain increases to 23% in 1983 and somewhat less in 1984. The technique we applied to work around the gaps is described by Kuhn^{16,17}. By choosing a particular set of evenly spaced frequencies the window function effects can be minimized (the 'swap' technique). Unfortunately, numerical experiments on synthetic data with this domain could not be completely 'dealised' and we've concluded that the domain is too sparse for this method (and probably any other). We finally settled on the statistical approach, described below, to search for *g*-modes.

We take as a null hypothesis that the 1983 and 1984 power spectra (Fig. 1) are the result of random uncorrelated normally distributed noise. If this is correct then the distribution of power from both years should be consistent with the power distribution generated from noise analysed in the same way as the data. Thus we generate fake data sets on the same domain as the actual data. The values are distributed normally from a distribution whose variance is scaled so that the mean fake power and actual power agree. The resulting cumulative power probability distributions are plotted in Fig. 3. The agreement is quite good if the anomalous peak at 1/6 day period in the 1984 data is ignored. The Kolmogorov-Smirnov test yields a useful quantitative description of the probability that both samples came from the same parent distribution. The K-S test is essentially a measure of the largest deviation between the two distributions¹⁸. Applying the test to the 1983 and 1984 data separately yields statistic values of 0.34 and 0.86 respectively, indicating that we should accept the null hypothesis. In short, we find no evidence of solar *g*-modes in oblateness power spectra of mean noise 0.4 milliarcsec².

Further, if the previously detected 160.01-min periodic signal is interpreted as evidence of an $l = 2$ *g*-mode then its amplitude has decreased from near 1 to 0.1 m s^{-1} since its detection in 1974. Unless a hitherto unknown damping mechanism is postulated this is inconsistent with the expected mode damping time of $\sim 40,000 \text{ yr}$ ¹². We believe it is more likely that the 160.01-min signal is not due to a solar *g*-mode.

This research was supported in part by the AFGL under contract number F19628-84-K-0024, and in part by the National Science Foundation.

Received 22 July; accepted 22 October 1985.

- Scherrer, P. H. *Mem. Soc. astr. ital.* **55**, 83-89 (1984).
- Isaak, G. R., Van der Raay, H. B., Palk, P. L., Roca Cortes, T. & Delache, P. *Mem. Soc. astr. ital.* **55**, 91-97 (1984).
- Frolich, C. & Delache, P. *Mem. Soc. astr. ital.* **55**, 99-106 (1984).
- Kotov, V. A., Severny, A. B. & Tsap, T. T. *Mem. Soc. astr. ital.* **55**, 117-122 (1984).
- Scherrer, P. H. & Wilcox, J. M. *Sol. Phys.* **82**, 37-42 (1983).
- Kuhn, J. R. *Mem. Soc. astr. ital.* **55**, 69-74 (1984).
- Hill, H. A., Bos, R. J. & Goode, P. R. *Phys. Rev. Lett.* **49**, 1794-1797 (1982).
- Kuhn, J. R., Libbrecht, K. G. & Dicke, R. H. *Astrophys. J.* **290**, 758-764 (1985).
- Libbrecht, K. G. & Kuhn, J. R. *Astrophys. J.* **277**, 889-895 (1984).
- Dicke, R. H. & Goldenberg, H. M. *Astrophys. J. Supp.* **27**, 131-182 (1974).
- Dicke, R. H., Kuhn, J. R. & Libbrecht, K. G. *Nature* **316**, 687-690 (1985).
- Kuhn, J. R. & Boughn, S. P. *Nature* **308**, 164-165 (1984).
- Christensen-Dalsgaard, C. D. & Gough, D. O. *Mon. Not. R. astr. Soc.* **198**, 141-171 (1982).
- Groth, E. J. *Astrophys. J. Supp.* **29**, 285-302 (1975).
- Grec, C., Fossat, E. & Pomerat, M. *Nature* **288**, 541-543 (1980).
- Kuhn, J. R. *Astr. J.* **87**, 196-201 (1982).
- Kuhn, J. R. *Solar Seismology from Space* (ed. Ulrich, R. K.) 283-289 (JPL, Pasadena, 1984).
- Hollander, M. & Wolfe, D. *Non-parametric Statistical Methods*, 219-228 (J. Wiley, New York, 1973).

Are the Falkland Islands a rotated microplate?

C. Mitchell*, G. K. Taylor†‡, K. G. Cox* & J. Shaw†

* Department of Earth Sciences, University of Oxford, Parks Road, Oxford OX1 3PR, UK

† Department of Geology, University College, PO Box 78, Cardiff CF1 1XL, UK

‡ Present address: Department of Geology and Physical Sciences, Oxford Polytechnic, Headington, Oxford OX3 0BP, UK

Here we report preliminary palaeomagnetic evidence from dolerite dykes on West Falkland which suggests that the Falkland Islands were rotated through $\sim 120^\circ$ during the early stages of the break-up and dispersal of the southern part of Gondwanaland. The rotation itself, and evidence of palaeolatitude, confirm the proposal made by Adie¹ in 1952 that the islands were formerly situated adjacent to the Transkei area of South Africa, where they formed the south-east corner of the Karoo basin with its adjoining Palaeozoic fold belts. The first stage of continental drift probably took place during the Jurassic, as Antarctica separated from southern Africa, the islands moving as a microplate to a position approximately 500 km south-east of present-day Cape Town. Subsequently, during the opening of the South Atlantic, the islands and the Falklands Plateau have drifted to their present position and undergone a further rotation of $\sim 60^\circ$.

Figure 1 outlines the geology of the Falkland Islands, and the inset shows the position they occupied before the opening of the South Atlantic in the Lower Cretaceous. This position is obtained by closing the South Atlantic by an anticlockwise rotation of the South American plate through 56.40° about a Euler pole at 46.75°N , 32.65°W , keeping the African plate fixed². This is one of the more straightforward plate tectonic reconstructions which is constrained by a wealth of geophysical and bathymetric data, seafloor magnetic anomalies, the orientation of transform faults and the geometrical fit of the opposing continental shelves of South America and Africa^{3,4}.

Table 1 Palaeomagnetic results

Sample code	Dyke	<i>n</i>	<i>R</i>	<i>k</i>	α_{95}	Dec.	Inc.
F30	A	6	5.62	12.2	19.9	16.0	61.0
F29	A	5	4.86	91.6	8.0	335.4	63.4
F38	A	6	5.97	203.5	4.7	323.1	53.8
F23	B	6	5.92	62.9	8.5	332.1	65.8
F32	A	4	3.70	14.4	25.0	21.8	70.7
F24	B	6	5.93	78.6	7.6	300.5	69.3
F36	C	7	6.79	28.6	11.5	336.3	39.0
F25	B	6	5.94	79.1	7.6	349.2	55.9
F41	D	6	5.89	46.2	10.0	159.5	-24.5*
F31	A	5	4.97	128.9	6.8	7.4	8.6*
F46	E	4	3.94	48.2	13.4	174.9	-54.7
F47	E	6	5.13	5.7	30.6	357.8	-81.5*
F45	E	7	6.10	6.8	25.6	246.1	-24.5*
F6	E	4	—	—	—	—	—*
Mean		9	8.73	29.5	9.6	342.8	61.1

Palaeomagnetic South Pole = 5.3°S , 287.2°E . $dp/dm = 11.3/14.8$, where dp and dm are the semi-axes of the oval of 95% confidence about the palaeomagnetic pole. *n*, The number of specimens; *k*, Fisher's precision parameter; α_{95} , the radius of the 95% confidence cone about the mean direction; dec. and inc., the declination and inclination, respectively. Positive (negative) inclinations indicate reverse (normal) polarity in the Southern Hemisphere. Samples F45 and F47 are total NRM only. The first 10 samples in the table are from four different north-south dykes designated A-D. The last four samples are baked sediments from the contact of an east-west dyke, E.

* Sample direction not included in the calculation of the mean.

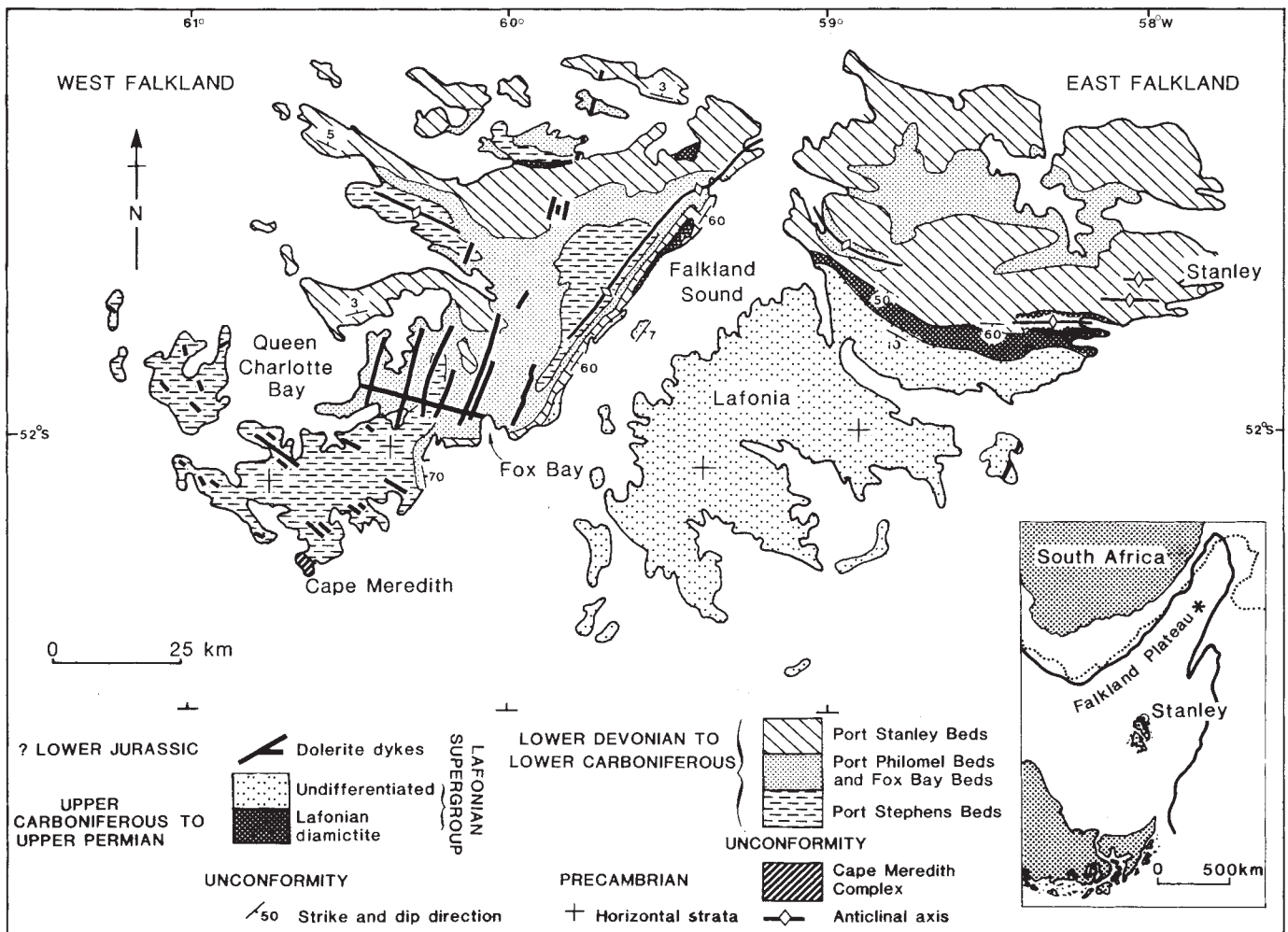


Fig. 1 Geological map of the Falkland Islands, after Greenway¹⁷. For purposes of comparison with South African geology, note that the Lafonian Supergroup correlates broadly with the Karoo, while the Port Stanley Beds, Fox Bay Beds and Port Stephens Beds correlate broadly with the South African units Witteberg, Bokkeveld and Table Mountain sandstone of the Cape Supergroup. Inset, position of the Falkland Islands and Falkland Plateau before the opening of the South Atlantic (Lower Cretaceous) (based mainly on Martin *et al.*²). The solid line marks the edge of the south American continental shelf; the dotted line marks that of South Africa. Asterisk shows the position of DSDP site 330 (ref. 14).

Nevertheless, before the development of plate tectonic theory, geologists sought to position the islands in reconstructions of Gondwanaland so that the main geological features matched those of neighbouring continents. For example, du Toit⁵ placed the islands to the west of Cape Town, in order to achieve continuity of the east-west fold belts of the Cape, Port Stanley and northern West Falkland in the Falklands and of the Sierra de la Ventana in Argentina. Adie's suggestion¹ that the islands had previously been situated at the east end of the Cape fold belt (the Zwartberg fold belt), offshore from present-day Transkei (Fig. 2), provided near-perfect correlation of Falklands and South African geological features by rotating the islands through 180° from north to south; he cited transport directions in the Dwyka and Lafonian diamictites (with movement from south to north in the Falklands) in support of the postulated rotation. Adie's reconstruction juxtaposes the 1,000-Myr-Natal basement⁶ and the Cape Meredith Complex in the Falkland Islands, which is of a similar age⁷; it also removes a problem inherent in the du Toit reconstruction by achieving continuity of the Karoo strata. The Adie reconstruction is much favoured by geological correlations but its validity depends on whether a substantial rotation of the Falkland Islands can be demonstrated.

During a field study in January 1985, C.M. and K.G.C. collected a number of magnetically orientated samples, which comprised 10 hypersthene-bearing dolerites from four different

north-south-trending dykes and four baked shales adjacent to a large olivine-bearing east-west dyke, of which, however, no *in situ* exposures were found. All specimens were collected in the area between Fox Bay and Queen Charlotte Bay in West Falkland (Fig. 1). The dykes have usually been assumed to be of the same age as the Karoo dolerites of South Africa; the one published age-determination⁸, from a dyke near Cape Meredith, yields a typical Karoo age (Lower Jurassic) of 192 ± 10 Myr by the K-Ar method. Each of our specimens provided a minimum of five 9-mm cylindrical samples for measurement, using the automated SQUID magnetometer at Cardiff⁹.

Most samples were demagnetized using alternating fields (AF), but a few were subjected to thermal demagnetization. The results were analysed by means of orthogonal and stereonet plots combined with principal-component analysis¹⁰. All 10 dolerite samples yielded stable coherent within-sample directions, but two samples (F41 and F31) clearly deviate from the mean of the others (Table 1). These two anomalous results may represent either poor orientation of the samples or the genuine recording of an intermediate geomagnetic field direction. The characteristic direction is generally stable in the range 15–70 mT, with randomly directed low-coercivity components removed on treatment to 15–20 mT, except samples F30 and F32 which were relatively unstable to both AF and thermal demagnetization, compared with the other samples. The AF demagnetization

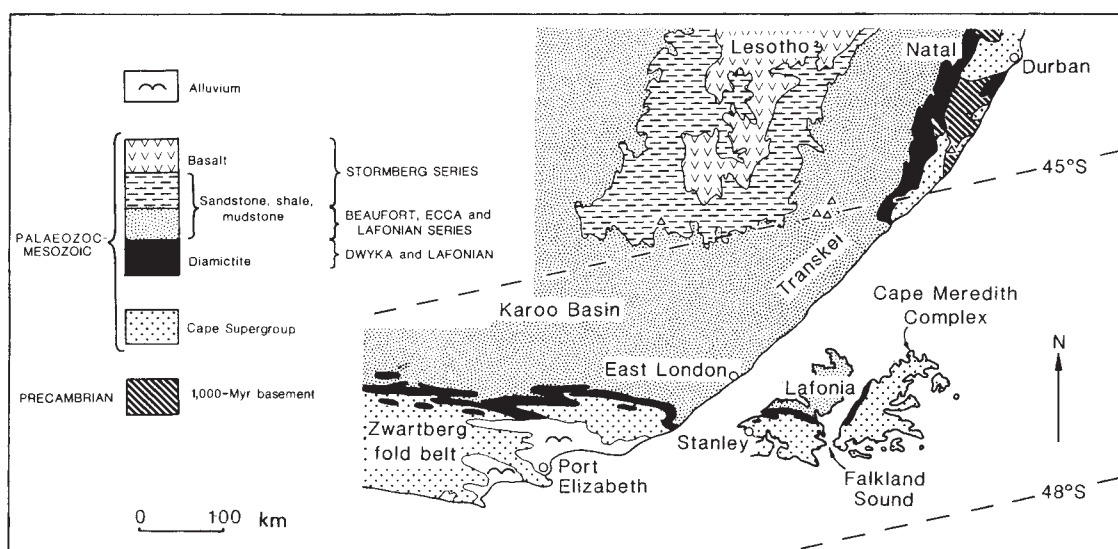


Fig. 2 Proposed reconstruction of the Falkland Islands relative to South Africa in the Lower Jurassic. This reconstruction is essentially similar to that of Aidie (see Fig. 2 of ref. 1). Palaeolatitude lines are based on McElhinny *et al.*¹¹. Δ , Sites from which palaeomagnetic data for Karoo dolerites were obtained.

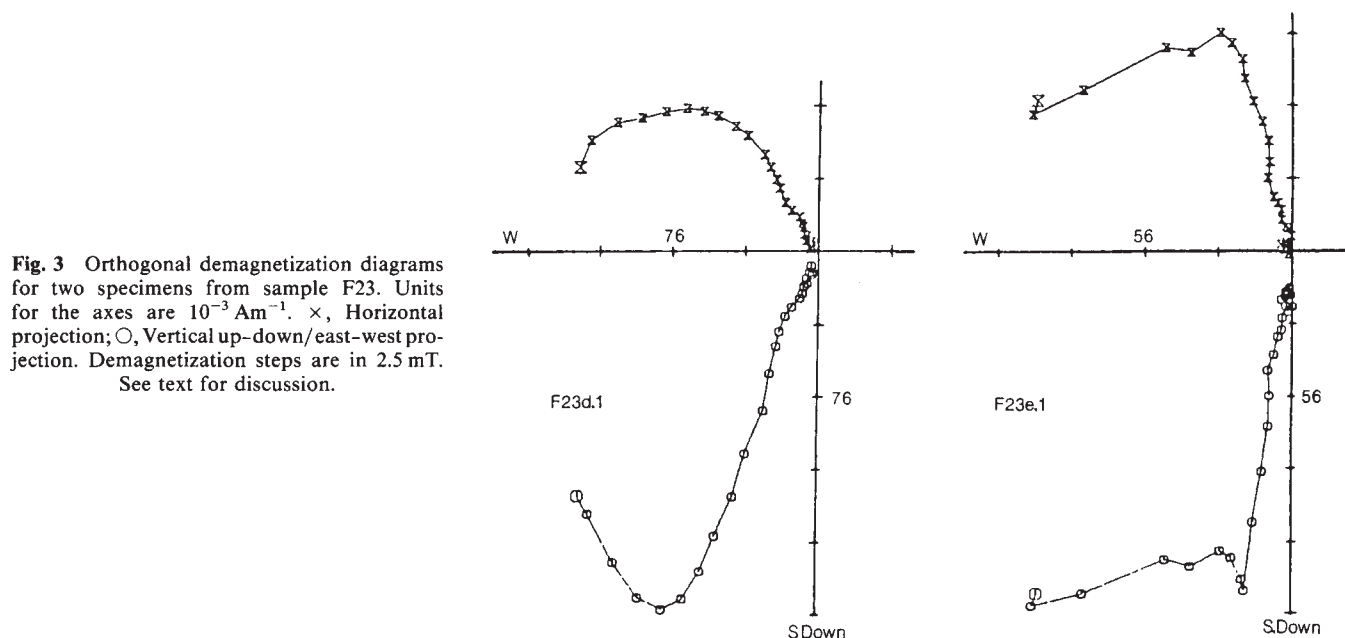


Fig. 3 Orthogonal demagnetization diagrams for two specimens from sample F23. Units for the axes are 10^{-3} A m^{-1} . $\times$, Horizontal projection; $\circ$, Vertical up-down/east-west projection. Demagnetization steps are in 2.5 mT. See text for discussion.

behaviour of two specimens from sample F23, a hypersthene-bearing north-south dyke, is illustrated in Fig. 3. These two specimens demonstrate the normal range of within-sample variability; in specimen F23d.1 there is overlap of the low-coercivity components, typical of most specimens, while in F23e.1 these components are discrete. In both cases the characteristic direction of the high-coercivity component is readily isolated.

Three of the four baked sediment samples have specimen total NRM (natural remanent magnetism) intensities of $<1.5 \times 10^{-9} \text{ A m}^2 \text{ kg}^{-1}$ which are unstable to demagnetization, although samples F47 and F45 do yield poorly defined total NRM directions. Sample F46, however, has specimen total NRM intensities of $2.5 \times 10^{-8} \text{ A m}^2 \text{ kg}^{-1}$ which are stable to AF demagnetization. The AF demagnetization characteristics and thermal stability up to 550–580 °C suggest titanomagnetite as the main magnetic phase present in all samples.

The antiparallel nature of the directions for the dolerites versus the baked sediments (F46, supported by F47 and F45) is indicative of a polarity change between emplacement of the north-south and east-west dykes. The sample mean direction

yields an equatorial pole position, and this is inconsistent with any data from southern Gondwanaland in Jurassic times. A rotation by a total of $180 \pm 10^\circ$ is needed to produce a palaeo-pole in the expected near-polar position. Further, the palaeolatitude derived from the inclination is $42 \pm 6^\circ \text{ S}$, precluding the conventional reconstruction position south of the Cape, which demands a palaeolatitude of $\sim 55^\circ \text{ S}$. On the other hand, palaeolatitudes derived for southern Africa from Karoo dolerites, admittedly from old and limited data¹¹ (Fig. 2), suggest a palaeolatitude for the preferred reconstruction position of the Falklands at $47 \pm 5^\circ \text{ S}$, which is consistent with our results. However, because of the small number of samples involved, our results are of a provisional nature and will require confirmation by more detailed studies of both Falklands and Transkei dolerites.

Perhaps the most obvious problem with our proposed reconstruction is the mechanism by which the islands have travelled to their present location. All reconstructions of southern Gondwanaland are problematical and authors differ widely in their opinions as to the precise fit. Recent studies^{12,13} have indicated that there are several microplates in the area, some

of which have experienced complex movements, for example the Ellsworth Mountains in Antarctica. We tentatively suggest that the Falkland Islands formed one such microplate which was rotated through 180° and moved south and west as Antarctica moved southward and away from Africa during the Jurassic. The islands were then carried to their present location by the opening of the South Atlantic from the Lower Cretaceous onwards. However, drilling at site 330 (see Fig. 1) on the Maurice Ewing Bank¹⁴ shows that the easternmost segment of the Falkland Plateau is composed of continental crust. Furthermore, Tarney¹⁴ thought it likely that the whole of the Falkland Plateau was composed of similar continental crust because of petrographical and geochemical similarities to the Cape Meredith Complex. However, the crust drilled at site 330 yields an age of 535 Myr¹⁵ hence cannot be correlated with the 1,000-Myr-old gneisses of the Cape Meredith Complex. Also, more recent geophysical surveys¹⁶ of the Falkland Plateau revealed that the Falkland Plateau Basin, which separates the Maurice Ewing Bank from the main plateau, is floored by oceanic or highly thinned continental crust. Ludwig¹⁶ aligned Mesozoic magnetic anomalies off South Africa with their equivalents just off the Maurice Ewing Bank and found that the bank probably occupied a position closer to the plateau than it does at present. He went on to suggest that the Falkland Plateau Basin may represent a zone of seafloor spreading or stretching and subsidence of continental crust during early rifting of the continents. Therefore, it now seems unlikely that the Falkland Plateau is a simple extension of the South American and South African continental crust, and we suggest that it may be a collage of microplates. If, as might be expected, all the microplates recognized in this

area experienced complex motions during the break-up and dispersal of southern Gondwanaland, this may explain why reconstructions of southern Gondwanaland are so problematical.

We thank Dr C. W. M. Swithinbank (British Antarctic Survey) for drawing our attention to the existence of the Falklands dolerites. Thanks are also due to Mr and Mrs A. McMullen for their hospitality at Fox Bay West. Field and laboratory work in Oxford were supported by NERC research grant GR3/5350 and by a travel grant from the Royal Society. NERC grant GR3/3901 supported the development of the super conducting magnetometer used for this work at Cardiff.

Received 12 July; accepted 28 October 1985.

1. Adie, R. J. *Geol. Mag.* **89**, 401-410 (1952).
2. Martin, K. A., Hartnady, C. J. & Goodlad, S. W. *Earth planet. Sci. Lett.* **54**, 293-305 (1981).
3. Bullard, E. C., Everett, J. E. & Smith, A. G. *Phil. Trans. R. Soc. A* **1088**, 41-51 (1965).
4. Le Pichon, X. & Hayes, D. E. *J. geophys. Res.* **76**, 6283-6293 (1971).
5. du Toit, A. L. *Publ. Carnegie Instn* **381** (1927); *Our Wandering Continents* (Oliver & Boyd, Edinburgh, 1937).
6. Nicolaysen, L. O. & Burger, A. J. *Sci. Terre.* **10**, 497-516 (1965).
7. Rex, D. C. & Tanner, P. W. G. in *Antarctic Geoscience* (ed. Craddock, C.) 107-110 (University of Wisconsin Press, 1982).
8. Cingolani, C. A. & Varela, R. in *Actas 6° Congreso Geológico Argentino Vol. 1* (Congreso Geológico Argentino, Buenos Aires, 1976).
9. Shaw, J., Share, J. A. & Rogers, J. *Geophys. J.R. astr. Soc.* **78**, 209-218 (1984).
10. Kent, J. T., Briden, J. C. & Mardia, K. V. *Geophys. J.R. astr. Soc.* **75**, 593-622 (1983).
11. McElhinny, M. W., Briden, J. C., Jones, D. L. & Brock, A. *Rev. Geophys.* **6**, 201-238 (1968).
12. Watts, D. R. & Bramall, A. M. *Nature* **293**, 638-640 (1981).
13. Longshaw, S. K. & Griffiths, D. H. *J. geol. Soc. Lond.* **140**, 945-954 (1983).
14. Tarney, J. *Init. Rep. DSDP* **36**, 893-921 (1977).
15. Beckinsale, R. D., Tarney, J., Darbyshire, D. P. F. & Humm, M. J. *Init. Rep. DSDP* **36**, 923-927 (1977).
16. Ludwig, W. J. *Init. Rep. DSDP* **71**, 281-293 (1983).
17. Greenway, M. E. *Br. Antarctic Surv. Sci. Rep.* No. 76 (1972).

Production of ¹⁰Be and ²⁶Al by cosmic rays in terrestrial quartz *in situ* and implications for erosion rates

K. Nishiizumi*, D. Lal†, J. Klein‡, R. Middleton‡ & J. R. Arnold*

*Department of Chemistry, B-017, and †Scripps Institute of Oceanography, A-020, University of California, San Diego, La Jolla, California 92093, USA

‡Tandem Accelerator Laboratory, Department of Physics, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

We present results of determinations of ¹⁰Be (half life = 1.6 Myr) and ²⁶Al (half-life = 0.705 Myr) produced by cosmic rays *in situ* in several terrestrial rock samples exposed at altitudes of 1-4 km. These experiments were designed to determine the feasibility of using quartz extracted from natural surfaces for studying continental weathering/erosion processes¹. Quartz is attractive as a target material for several reasons. Its low aluminium concentration allows measurement of ²⁶Al after exposures as short as 10³ yr; its integrity minimizes the possibility of contamination by rain with ¹⁰Be produced in the atmosphere; and its ubiquity ensures that it is found in a wide variety of geological settings. However, the cosmic-ray production rates of ²⁶Al and ¹⁰Be even at mountain altitudes are low^{2,3}, only 10-100 atoms g⁻¹ yr⁻¹, with the consequence that earlier attempts^{4,5} to determine *in situ* produced ³⁶Cl and ²⁶Al in rocks were not continued. Recent development of accelerator mass spectrometry (AMS) however, has made the measurement of 10-20g samples feasible. The results presented here demonstrate the feasibility of quantitatively measuring ¹⁰Be and ²⁶Al produced *in situ* by cosmic rays in quartz and the possible applications of ¹⁰Be and ²⁶Al as a pair for studying continental weathering/erosion processes.

Table 1 Sample description

Sample	Site	Elevation (m)	Weight of quartz analysed (g)	Al content of quartz (p.p.m.)	Be carrier added (mg)
ALH	Allan Hills, Antarctica	2,050	13.09	467	1.49
Q-1	Anza Borrego, California	1,140	20.22	4.9 × 10 ⁴	1.37
Q-L	Anza Borrego, California	1,200	22.18	100	1.50
KKS-1	Leh, Ladakh	4,000	17.29	52*	1.50
KKS-3	Kinnaur, Himachal Pradesh	3,400	16.39	289	1.50

* Intrinsic Al content of the purified quartz grains; an amount equivalent to 0.53 mg Al was added for AMS measurement.

The rock samples analysed are listed in Table 1. Sample ALH, which is quartzite (determined by thin section), was collected at the peak of Allan Hills, Antarctica (76°42' S, 150°31' E), elevation 2,050 m, by one of us (K.N.) during the 1983-84 field season of Antarctic search for meteorites. Allan Hills are located about 230 km north-west of McMurdo station. The large blue ice area (Allan Hills ice field) is stagnated by the Allan Hills. More than 2,000 meteorites have been found on the ice field and contiguous areas during the past 10 yr. At present, the Allan Hills are not covered with ice because of strong winds which prevent net snow accumulation in winter; in summer there is no precipitation. It would be useful to determine when Allan Hills lost their ice sheets. The sample was collected from the surface of a flat-top area, ~200 m north of the glaciological survey station⁶. The Q-1 and Q-L samples were collected from Anza Borrego, California (33°12' N, 116°27' W), ~100 km east of San Diego by A. E. Engel and us. The samples were collected from the flat tops of large rocks (~5 m in diameter) located at

elevations of 1,140 m (Q-1) and 1,200 m (Q-L). The ^{40}K - ^{40}Ar age of the Borrego rocks is ~ 90 Myr. Sample KKS-1 was collected from a quartz vein intruding Indus Flysch near Kiari, Kashmir, India (34°N , 78°E), at an elevation of 4,000 m. KKS-3 was collected from quartz-kyanite veins intruding Kyanitesillimanite schists, Himachal Pradesh, India (32°N , 78°E), elevation 3,400 m.

Quartz was extracted from the rocks primarily by using the higher reactivity of feldspars with HF (details will be presented elsewhere). The chemical procedure used for extraction of Be and Al was essentially the same as in previous experiments for meteoritic and lunar samples⁷. The ^{10}Be and ^{26}Al measurements were performed on the University of Pennsylvania FN Tandem Van de Graaff accelerator^{8,9}.

As the ratio $^{10}\text{Be}/^9\text{Be}$ in commercially available beryllium¹⁰ is $\sim 1 \times 10^{-14}$, we prepared a ^9Be carrier from a beryl (supplied by B. Dassanacharya). The measured $^{10}\text{Be}/^9\text{Be}$ and $^{26}\text{Al}/^{27}\text{Al}$ ratios and the ^{10}Be and ^{26}Al concentrations in the quartz samples are shown in Table 2. The $^{10}\text{Be}/^9\text{Be}$ and $^{26}\text{Al}/^{27}\text{Al}$ ratios of the samples were normalized to standards⁷ after blank corrections. Our beryl derived Be carrier has a $^{10}\text{Be}/^9\text{Be}$ ratio $< 3 \times 10^{-15}$, corresponding to $< 2 \times 10^5$ atoms $^{10}\text{Be}/\text{mg Be}$.

Table 2 shows that the ^{10}Be and ^{26}Al produced *in situ* by cosmic rays can be measured easily in natural quartz samples of 10–20 g size. And, in fact, measurement of ^{26}Al produced *in situ* on Earth is as easy as in extraterrestrial samples if the ^{27}Al concentration is < 300 p.p.m.

Figure 1 shows the theoretical saturation values¹ plotted for ^{10}Be and ^{26}Al produced in quartz at the Earth's surface. The ^{10}Be is caused by nuclear spallation of Si and O, and the ^{26}Al is caused by nuclear spallation of ^{28}Si and negative mu-meson capture in ^{28}Si . Other contributions to the production of ^{26}Al and ^{10}Be from *in situ* radioactivity are negligible. The samples KKS-1 and KKS-3 are not surface samples; they were collected from quartz veins to obtain samples with low Al content. Figure 1 shows the ^{10}Be data for the three surface samples. These samples have concentrations lower than secular equilibrium values, corresponding to 2×10^4 – 1.5×10^6 yr of effective irradiation. As the samples are not at the surface secular-equilibrium concentration value, they must have been shielded by some overburden of material for an appreciable fraction of the flux-integrating period of these isotopes, $\sim 10^7$ yr. This overlying material could have been removed either all at once or gradually. If the samples had been covered by a thick ice sheet, a sedimentary deposit, or a layer of rock which was removed during the retreat of a glacier, the time needed to remove this cover could have been short ($< 10^4$ yr). If the overburden was removed by erosional weathering, the process could have been continuous, and the shielding of these samples could have been reduced gradually. The result is that the concentration of these radionuclides can be used to place upper limits on the rate of erosion (weathering) and lower limits on the amount of time that the samples have not been covered with an appreciable layer of either rock or ice. Such estimates depend on a knowledge of

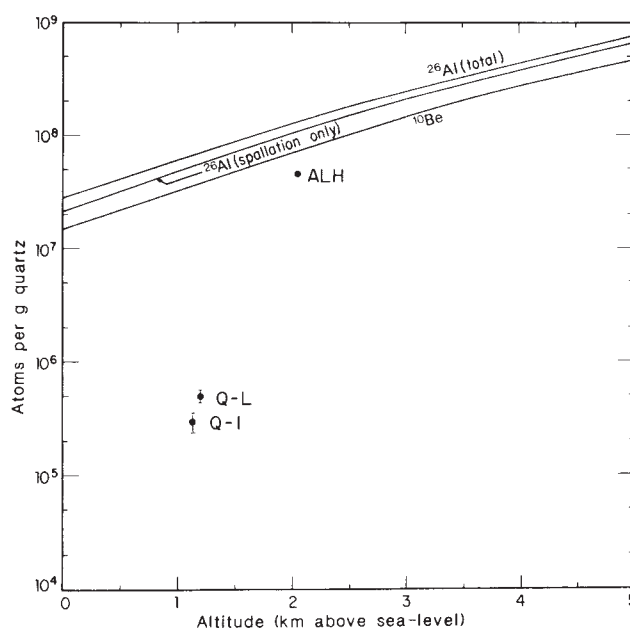


Fig. 1 Theoretically estimated *in situ* saturation values of ^{10}Be and ^{26}Al in quartz, based on production rates in ref. 1. Values are shown as a function of altitude for geomagnetic latitude (50 – 90°). ^{10}Be is produced in nuclear spallation of Si and O, and ^{26}Al from spallation of Si and negative mu-meson capture of ^{28}Si . The total production rate of ^{26}Al (spallation + capture) is shown as is the measured concentrations of ^{10}Be in three surface exposed rocks.

the rates of production of these isotopes, and on the model adopted to explain the exposure history of the samples.

Next, we briefly consider the expected isotope concentrations in terms of a simple exposure history model which occurs without change in the altitude of the sample. The production rate in the rock, $P(t)$ is time dependent because of change in the rock geometry caused by erosion of overlying ice/sediment. If the production rate of an isotope at the surface of a flat rock is P_0 , then the expected production rate $P(x)$ at x cm depth is:

$$P(x) = P_0 e^{-\rho x / \Lambda} \quad (1)$$

where Λ is the absorption mean-free-path (~ 150 g cm^{-2}) and ρ is the density of the rock. If the variation in x with time is known, the production rate at the test point ($x = 0$) for all past times can be calculated. The expected concentration of an isotope C , at the present rock surface is given by:

$$C_s(x=0) = \int_0^\infty P(t) e^{-\lambda t} dt \quad (2)$$

where λ is the disintegration constant of the isotope; and time, t , is reckoned backwards. For the simple case of a constant

Table 2 Measured ^{10}Be and ^{26}Al concentrations in quartz samples

Sample	$^{10}\text{Be}/^9\text{Be}$ ($\times 10^{-15}$)	^{10}Be (10^6 atoms g^{-1})	$^{26}\text{Al}/^{27}\text{Al}$ ($\times 10^{-15}$)	^{26}Al (10^6 atoms g^{-1})	$^{10}\text{Be}/^{26}\text{Al}$ (atom ratio)
ALH	$(6.00 \pm 0.24) \times 10^3$	45.0 ± 1.8	$(1.93 \pm 0.19) \times 10^4$	201 ± 20	0.224 ± 0.024
Q-1*	65 ± 14	0.29 ± 0.06	—	—	—
Q-L	110 ± 11	0.497 ± 0.050	$(1.48 \pm 0.16) \times 10^3$	3.31 ± 0.36	0.150 ± 0.022
KKS-1†	14.2 ± 1.7	0.082 ± 0.010	107 ± 19	0.207 ± 0.037	0.396 ± 0.086
KKS-3‡	4.8 ± 1.9	0.028 ± 0.011	$8.2^{+7.0}_{-4.4}$	$0.054^{+0.052}_{-0.029}$	$(0.03\text{--}1.0)$

* ^{26}Al could not be measured in this sample because of the high intrinsic aluminium content of the sample (see Table 1).

† These are not surface samples (see text); no estimates can be made on their shielding.

‡ The errors cited in the ^{26}Al values are the inverse Poisson estimates for three counts.

erosion ($dx/dt = \varepsilon$) the steady-state isotope concentration is given by:

$$C_{s(x=0)} = P_0 / (\lambda + \rho\varepsilon/\Lambda) \quad (3)$$

Inversion of equation (3) allows the deduction of the model erosion rates assuming that the sample is in secular equilibrium. An estimate based on another isotope of different half-life can be used to validate the erosion model adopted. In favourable cases, the ratio of concentrations of two isotopes can be used to determine the erosion rate, but if $\rho\varepsilon/\Lambda$ is $\ll \lambda$, the ratio can lead to erroneous estimates due to uncertainties in isotope production rates. If ε is too large, then isotopic concentrations are too low to measure. In practice, erosion rates $< 10^{-2}$ cm yr $^{-1}$ are easily measurable.

There are several advantages of using ^{10}Be and ^{26}Al , as opposed to ^{36}Cl , in this application. The production of ^{26}Al and ^{10}Be results from spallation of Si and O, the principal constituents of quartz. The production of ^{36}Cl is the result of spallation of several minor constituents, such as Ca, Ti, and the capture of thermal neutrons by ^{35}Cl . Furthermore, some of the chlorine in the rock is in a mobile form and can be exchanged in interactions with seepage water. Consequently, the ^{36}Cl production rates have to be determined for each rock studied. It is unlikely that the mineral phases which are best for the measurement of ^{36}Cl will also be suitable for measurements of ^{26}Al or ^{10}Be . In particular, minerals with sufficient concentration of target materials for the production of ^{36}Cl are unlikely to be low enough in ^{27}Al for ^{26}Al measurement. In addition, minerals with enough Ca and Ti for ^{36}Cl production are often easily altered during weathering, increasing the possibility of contamination from atmospheric sources of ^{10}Be and ^{36}Cl (from the bomb pulse).

We will discuss the implications of the results in Table 2 in view of the above simple steady-state erosional model and the theoretical ^{10}Be , ^{26}Al production rates in quartz, for samples ALH, Q-1 and Q-L only; the shielding depths of the two KKS samples are unknown, so that the absolute concentrations of ^{10}Be and ^{26}Al in terms of erosion rates cannot be considered.

The $^{10}\text{Be}/^{26}\text{Al}$ production ratios for 1–5 km altitude lie in the range 0.24–0.28 (Fig. 1); the atom ratios $^{10}\text{Be}/^{26}\text{Al}$ in rock samples should, therefore, be between 0.25 and 0.5, that is between the ratio at inception of production and the ratio at secular equilibrium. The measured values of the ratio do fall within this range for all samples except Q-L, which is lower than the lowest expected, by a factor of 1.7. The measured ratio in ALH is close to theoretical ratio at production which is surprising considering that the ^{10}Be concentration is high, corresponding to an effective period of irradiation (concentration/production rate at surface) of ~ 1.5 Myr, and one would expect a value of ~ 1.3 times the production ratio. The results of ALH and Q samples can be made consistent if one assumes a value for the $^{10}\text{Be}/^{26}\text{Al}$ ratio at production of ~ 0.15 – 0.17 . This would imply either that we¹ overestimated the ^{10}Be production rate or that we underestimated ^{26}Al production by a factor of ~ 1.5 . As the ^{26}Al concentration of the ALH sample is higher than the theoretical value, we probably underestimated the ^{26}Al production. Uncertainties in the estimation of production rates of ^{10}Be and ^{26}Al are at least $\pm 20\%$ and $\pm 30\%$ on the ratio. The disagreement is, therefore, not surprising.

Despite the uncertainties in production rates, we can obtain fairly meaningful model values of erosion rates, based on the observed concentrations of one of the isotopes. By partial differentiation of equation (3), we obtain:

$$(d\varepsilon/\varepsilon)/(dP_0/P_0) = 1/(1 - \lambda T) \quad (4)$$

where T is the effective irradiation period, $C_{x=0}/P_0$. Equation (4) shows that estimated rates of erosion are less sensitive to uncertainties in production rates for longer-lived isotopes, that is smaller λT . The effective irradiation periods (T) based on the ^{10}Be concentration for samples Q-1, Q-L and ALH are 1.75×10^4 ,

2.8×10^4 and 1.3×10^6 yr, respectively. These values correspond to erosion rates of 2.8×10^{-3} , 1.7×10^{-3} and 1.7×10^{-5} cm yr $^{-1}$. From equation (4), we deduce that a $\pm 30\%$ uncertainty in ^{10}Be production rate will lead to uncertainties of ± 30 , ± 30 and $\pm 68\%$ in the erosion rates in these three cases.

Our results clearly demonstrate the feasibility of measuring ^{10}Be and ^{26}Al in quartz. ^{26}Al determinations require mineral phases low in Al so that the $^{26}\text{Al}/^{27}\text{Al}$ ratio is high enough to measure. ^{10}Be studies can be made in any mineral as long as it can be established that it does not absorb rainwater-scavenged atmospheric ^{10}Be , which we designate as the 'garden-variety' ^{10}Be to distinguish it from that produced *in situ*. Once erosional models have been checked by studies of several isotopes (for example, ^{14}C , ^{36}Cl , ^{26}Al , ^{10}Be) measured in the same sample, large-scale synoptic erosional studies of land masses can be made based on ^{10}Be concentrations in abundant suitable rock minerals. These techniques can also be used to measure exposure times in surfaces in which the erosion rate is sufficiently low, or even to date volcanic flows, if suitable mineral phases can be isolated. This approach may therefore be a method of obtaining quantitative data on denudation of rock surfaces, for which no techniques are available.

Our present results show the potential of using ^{10}Be and ^{26}Al produced *in situ* by cosmic rays for quantifying continental erosion rates and other processes; erosion rates are primarily based on estimates of the transport of detrital sediments and dissolved elements to oceans from continental runoff. It is clear that better estimates of the isotope-production rates are needed and checks of erosional history with other isotopes produced by cosmic rays (stable and radioactive) would be helpful.

We thank A. L. Engel and E. L. Winterer for discussions and K. K. Sharma for providing the high-purity quartz samples KKS-1 and KKS-3. We thank F. Kirchner for typing the manuscript. This work was supported by NASA grant NAG 9-33; NSF grant Phy 82-13598, and Cal Space grant CS-04-85.

Received 2 July; accepted 11 October 1985.

1. Lal, D. & Arnold, J. R. *Proc. Ind. Acad. Sci.* **94**, 1–5 (1985).
2. Lal, D. & Peters, B. *Handbuch der Physik*, XLVI/2, 551–612 (Springer, Berlin, 1967).
3. Jha, R. & Lal, D. *Proc. 2nd Symp. on Natural Radiation Environment* (eds Vohra, K. G., Pillai, K. C., Mishra, U. C. & Sadasivan, S), 629–635 (Wiley Eastern Press, New Delhi, 1981).
4. Davis, R. & Schaeffer, O. A. *Annls N.Y. Acad. Sci.* **61**, 107–121 (1955).
5. Hampel, W. *et al. J. geophys. Res.* **80**, 3757–3760 (1975).
6. Nishio, F. & Annexstad, J. O. *Mem. Natn. Inst. Polar Res., Spec. Iss.* **15**, 13–23 (1979).
7. Nishiizumi, K. *et al. Earth planet Sci. Lett.* **70**, 157–163; 164–168 (1984).
8. Klein, J. *et al. Nucl. Instrum. Meth.* **193**, 601–616 (1982).
9. Middleton, R. *et al. Nucl. Instrum. Meth.* **218**, 430–438 (1983).
10. Middleton, R. *et al. Nucl. Instrum. Meth.* **B5**, 511–513 (1984).

Thermal erosion by komatiites at Kambalda, Western Australia and the genesis of nickel ores

D. I. Groves*, E. A. Korkiakoski*, N. J. McNaughton*, C. M. Leshner† & A. Cowden‡

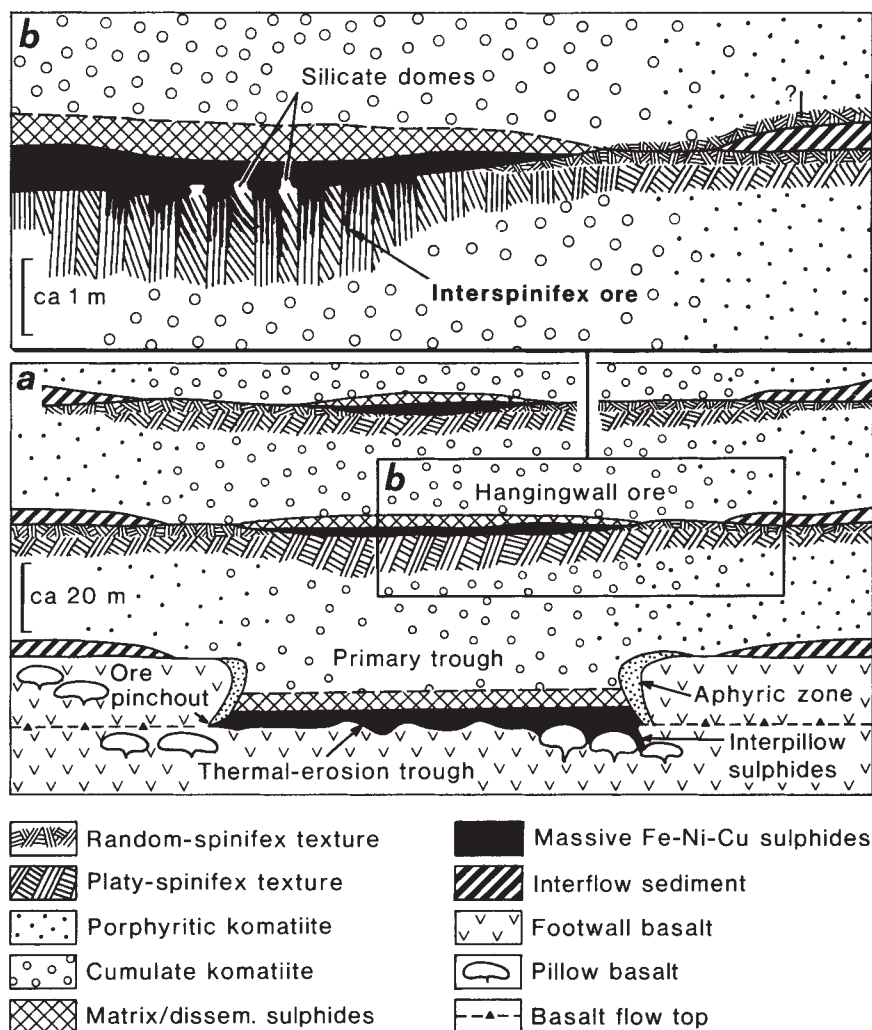
* Department of Geology, University of Western Australia, Nedlands 6009, Australia

† Department of Geology, The University of Alabama, University, Alabama 35486, USA

‡ Kambalda Nickel Operations, Western Mining Corporation Ltd, Kambalda 6442, Australia

Huppert *et al.*¹ calculated that komatiites flow turbulently, transfer heat efficiently to their substrate, and thus potentially produce thermal-erosion channels in sequences onto which they were erupted. Their suggestion that komatiite-hosted (Kambalda-type²) nickel ores reside in such channels has led to resurgence of interest in volcanic models for ore emplacement^{3–5} and caused controversy^{6,7}. Such models have important implications, not only to ore-genetic studies but also to interpretation of the geochemistry

Fig. 1 Schematic representation (not to scale) of: *a*, Relationships in the lower part of the Kambalda komatiite pile emphasizing embayment (trough) structures and showing hanging-wall ores (adapted from ref. 23); and *b*, relationships between massive ores, interspinifex ores and komatiite flow-tops at Lunnon Shoot.



of komatiites⁸ and their Sm-Nd systematics^{9,10}. Here we demonstrate that interspinifex Fe-Ni-Cu sulphide ores, which underlie some hanging wall massive sulphide ores at Kambalda the site of one of the world's major nickel deposits, formed through thermal erosion of underlying komatiite flows and possibly also sediments, providing proof of thermal erosion. However, we caution against the general application of thermal-erosion models to the eruption of komatiites. It seems that significant thermal erosion occurred rarely, and only where basal concentrations of highly thermally conductive sulphide liquid were present at the base of localized lava channels. Isotope data^{11,12} and theoretical considerations¹³ suggest that the komatiites were contaminated by crustal rocks before eruption, although there is limited evidence that localized contamination, including sulphide assimilation⁴, may also have occurred below lava channels localized in pre-existing embayments or troughs.

Despite recent predictions that komatiites cause significant thermal erosion of their substrates¹, there has been no unequivocal supporting field evidence. This has led to suggestions that real komatiites may be less voracious than mathematical counterparts⁶. Therefore, we have tested the models using the well-documented Kambalda nickel deposits^{2,3,5}. First, we present evidence for the role of thermal erosion in the formation of very localized interspinifex ores, and then we discuss its wider relevance.

Interspinifex ore is a very minor component of hanging wall ores at Lunnon, Fisher and Hunt Shoots, Kambalda²; its situation is shown schematically in Fig. 1. Basically, it comprises Fe-Ni-Cu sulphides interstitial to pseudomorphs after platy

olivine spinifex. This ore type occupies shallow depressions in the top of the lowermost flow and is physically contiguous with overlying sulphide ore, which forms the base of the second flow in the komatiite pile. Replacement of original interstitial glass and/or pyroxene by Fe-Ni-Cu sulphides is required by (1) the almost perfect preservation of spinifex texture, and (2) transitions from sulphides to the mesostasis adjacent to pseudomorphs after olivine plates. The best interpretation is that glass and pyroxene in the spinifex-textured top of the lower flow were selectively melted by the overlying sulphide-bearing komatiite flow, and the generated silicate liquid was displaced upwards by the denser sulphide liquid. The critical evidence for such an origin includes: (1) the similarity of chemical compositions (Ni, Cu, Co, PGE) of spinifex-textured ore and overlying massive ore¹⁴; (2) the occurrence of magmatic ferrochromites in the spinifex-textured ores, and chromite zones on silicate-sulphide interfaces as in magmatic ores¹⁵; (3) the presence of randomly buckled olivine blades, consistent with emplacement of hot sulphide liquid between them, and (4) the occurrence of small chromite-rimmed, silicate-rich domes, consistent with the composition of interpreted segregations of displaced silicate liquid, at the base of overlying massive ore. None of these features is consistent with metamorphic-hydrothermal replacement, the only alternative origin.

Interspinifex ore was formed at temperatures below the olivine liquidus¹⁶ but above the sulphide liquidus¹⁷. The fact that virtually all interspinifex ore has platy-spinifex texture rather than random-spinifex texture, which typically occurs at the top of komatiite flows at Kambalda¹⁸, suggests thermal erosion of the

uppermost part of the underlying flow before formation of the interspinifex ore. Stratigraphical correlations with komatiite-komatiite contacts along strike, combined with measurements of the shallow depressions occupied by interspinifex ore, suggest a maximum of 1 m of erosion of komatiite before formation of that ore (Fig. 1b). Thus, a *prima facie* case for thermal erosion is established. The interflow sediment, present along strike, may also have been eroded but there is no direct evidence.

The significance of these observations to the applicability of thermal-erosion models, in general^{1,6,7}, can be assessed through (1) relationships along strike and down dip from interspinifex ores, and (2) consideration of observational data for more typical basal (contact) ores² in combination with accumulated petrological, geochemical and isotopic data from Kambalda komatiites.

Relationships in localized interspinifex ore environments indicate that thermal erosion is virtually restricted to some portions of the contact occupied by massive sulphide ores (Fig. 1). Normally, the upper komatiite flow conformably overlies interflow sulphidic sediments with little evidence for interaction, or directly overlies fine-grained random spinifex-textured komatiite. Thin amphibole-chlorite rich assemblages occur at the base of the upper flow in places¹⁹, and probably represent former chilled margins¹⁸. Thus field evidence from hanging wall ore-environments at Kambalda indicates that thermal erosion of komatiite occurred locally only where pools of sulphide liquid had already accumulated, presumably caused by the order-of-magnitude higher thermal conductivity of the sulphide liquid²⁰ relative to komatiite liquid^{21,22}, and its lower liquidus temperature¹⁷. There is no definitive evidence to indicate whether the massive sulphides were purely magmatic liquids, or whether their occurrence marks the position of lava channels⁴, in which more prolonged throughput of lava was capable of assimilating sulphide sediments and generating sulphide liquid below which thermal erosion of komatiite continued.

Observations on basal ore environments at Kambalda favour a primary volcanic/topographical control on embayments and localized lava channels²³ rather than initial formation by thermal erosion. Interpretation is hindered by subsequent, commonly severe, metamorphic alteration of the ultramafic rocks combined with structural modification^{2,23}. Despite this, the komatiites within the lava channels are texturally and geochemically distinct from flanking flows²³, which have conformable contacts with underlying sulphide sediments over >90% of the total contact surface. Komatiites within the lava channels show evidence for prolonged flow-through of magma, not shown by flanking flows. Evidence from Kambalda that confining embayments were primary volcanic features before komatiite eruption includes: (1) stratigraphical control of some embayments whose base (the base of the initial komatiite flow) coincides with the flow top of the penultimate basalt flow²³, (2) the presence of probable aphyric margins on komatiites around some embayments²³, and (3) the elongated form of most major embayments parallel to the interpreted fault-bounded margins of the volcanic basin²⁴. The presence of unassimilated or partly assimilated sediments in the similar embayments at Scotia and at Wannaway³ indicate that those embayments also predate sulphide ore and komatiite emplacement.

Structural and metamorphic modification of ore environments inhibits unequivocal interpretation. However, there are second-order irregularities within major embayments that could possibly be formed by thermal erosion. These include: (1) spaced 1–2 m convex-upward, projections of basalt into massive ore (such as Long and Durkin Shoots)²³; (2) small elliptical depressions confining Fe–Ni–Cu ores (such as Ken Far East, Juan East); (3) widespread, structurally modified, lateral tongues of massive sulphide in basalts in ore pinchouts (Fig. 1a); and (4) interpillow Fe–Ni–Cu sulphides which may well be representative of thermal erosion. The unusual silicate gangue mineralogy of massive ore^{25,26} could also be partly caused by melting of basal chills

and/or basalt, and preservation of so-formed immiscible silicate melt in sulphides below overlying crystallized olivine cumulates (compare with silicate domes associated with interspinifex ores). These features indicate the possibility that localization of lava channels and basal sulphide liquids in original volcanic/topographical embayments focused thermal erosion and modified the original embayments.

Several lines of evidence suggest that Kambalda komatiites are contaminated; (1) Sm–Nd (refs 9, 10) and Pb–Pb (ref. 12) or U–Pb (ref. 11) isotope studies indicate that Sm–Nd 'isochrons' are mixing lines between komatiites and older crustal components; (2) Pb–Pb studies of iron sulphides from nickel ores and adjacent sulphide sediments suggest mixing of mantle and older crustal lead²⁷; (3) the similarity of S/Se ratios and $\delta^{34}\text{S}$ values of nickel ores and sulphide sediments^{4,25} and anomalously high Zn chromites in mineralized komatiites^{15,27}. Several of these geochemical parameters indicate that contamination affected both the basal mineralized komatiite(s) and the entire komatiite pile. These include: (1) the tight grouping of both komatiites and basalts on Sm–Nd mixing lines^{6,9}; (2) the occurrence of >3,000 Myr xenocrystic zircons in overlying komatiitic basalts¹¹; and (3) chalcophile-element depletion²⁸ and occurrence of Zn-rich chromites¹⁵ throughout the Kambalda komatiite pile. Collectively, these data suggest that sulphide liquids separated from komatiites that were contaminated during ascent through older continental crust, as predicted theoretically¹³. However, the similarity of S/Se ratios and $\delta^{34}\text{S}$ values of nickel ores and adjacent sulphide sediments^{4,25} plus the fact that lead isotope compositions of iron-sulphides from nickel ore could represent mixtures of mantle lead and lead from the sulphide sediments²⁷, suggests that assimilation of sulphide sediments contributed to sulphide liquids in at least some komatiites.

There is unequivocal evidence from hanging wall ore environments at Kambalda for localized, shallow thermal erosion channels in early komatiites (and possibly sediments) underlying subsequent komatiite flows containing basal sulphide liquid pools. Conduction of heat from the overlying turbulently convecting komatiite through the highly thermally conductive sulphides seems to have been the controlling process. In the major basal (contact) ore environments, however, primary major embayments were volcanic/topographical features. There is some evidence for melting of the basalt substrate by thermal erosion only beneath sulphide-liquid pools at the base of lava channels confined by the embayments. Some sulphide liquid probably formed as a result of initial thermal erosion of sulphidic sediments. Such assimilation can explain some of the contamination shown by komatiites, but other parameters suggest contamination during magma ascent. Our study suggests that komatiites were erosive, but were more lethargic than previously suggested. It stresses the role of highly conductive sulphides, associated with lava channels, in thermal erosion of komatiitic and basaltic substrates by komatiite lavas.

We thank J. J. Gresham of Kambalda Nickel Operations for access to the Kambalda deposits, and several other Kambalda geologists for documentation and discussions of Kambalda geology. We also acknowledge UWA colleagues, particularly N. Dahl and P. Parker, and CSIRO staff, especially R. E. T. Hill, for useful discussions. E.K. was sponsored by the Australia-European Awards Programme and partly funded by Kambalda Nickel Operations.

Received 7 August; accepted 21 October 1985.

- Huppert, H. E., Sparks, R. S. J., Turner, J. S. & Arndt, N. T. *Nature* **309**, 19–22 (1984).
- Gresham, J. J. & Loftus-Hills, G. D. *Econ. Geol.* **76**, 1373–1416 (1981).
- Leshner, C. M., Arndt, N. T. & Groves, D. I. *Sulphide Deposits in Mafic and Ultramafic Rocks* (eds Buchanan, D. L. & Jones, M. J.) 70–80 (Institution of Mining and Metallurgy, London, 1984).
- Leshner, C. M. & Groves, D. I. *Geology and Formation Conditions of Copper Deposits* (eds Naldrett, A. J., Ridge, J. D., Vokes, F. M., Genkin, A. D. & Sillitoe, R. H.) (Springer, Berlin, in the press).
- Gresham, J. J. *Geology and Formation Conditions of Copper Deposits* (eds Naldrett, A. J., Ridge, J. D., Vokes, F. M., Genkin, A. D. & Sillitoe, R. H.) (Springer, Berlin, in the press).

6. Claoue-Long, J. C. & Nesbitt, R. W. *Nature* **313**, 247 (1985).
7. Huppert, H. E., Sparks, R. S. J. & Arndt, N. T. *Nature* **13**, 247-248 (1985).
8. Nisbet, E. G. *Nature* **309**, 14-15 (1984).
9. Claoue-Long, J. C., Thirlwall, M. F. & Nesbitt, R. W. *Nature* **307**, 697-701 (1984).
10. Bickle, M. J. *Nature* **312**, 702-703 (1984).
11. Compston, W., Williams, I. S., Campbell, I. H. & Gresham, J. J. *Earth planet. Sci. Lett.* (in the press).
12. Chauvel, C., Dupre, B. & Jenner, G. A. *Earth planet. Sci. Lett.* **74**, 315-324 (1985).
13. Huppert, H. E. & Sparks, R. S. J. *Earth planet. Sci. Lett.* **74**, 371-386 (1985).
14. Woolrich, P., Cowden, A. & Giorgetta, N. E. *Econ. Geol.* **76**, 1629-1644 (1981).
15. Groves, D. I., Barrett, F. N., Binns, R. A. & McQueen, K. G. *Econ. Geol.* **72**, 1224-1244 (1977).
16. Arndt, N. T. *Yb. Carnegie Instn. Wash* **75**, 555-562 (1976).
17. Craig, J. R. & Kuilerud, G. *Econ. Geol. Monogr.* **4**, 344-358 (1969).
18. Ross, J. R. & Hopkins, G. M. F. *Economic Geology of Australia and Papua New Guinea, I. Metals* (ed. Knight, C. L.) 100-121 (Australian Institute of Mining and Metallurgy, Melbourne, 1975).
19. Bavinton, O. A. thesis, Australian National Univ. (1979).
20. Williams, R. K., Veeraburns, M. & Philbrook, W. O. *AIME Metall. Trans.* **3**, 255-260 (1972).
21. Jaeger, J. C. *Basalts: The Poldervaart Treatise on Rocks of Basaltic Composition* (eds Hess, H. H. & Poldervaart, A.) 503-536 (Wiley, New York, 1968).
22. Bickle, M. J. *Komatiites* (eds Arndt, N. T. & Nisbett, E. G.) 479-494 (Allen and Unwin, London, 1982).
23. Leshner, C. M. thesis, Univ. Western Australia (1983).
24. Groves, D. I., Leshner, C. M. & Gee, R. D. *Sulphide Deposits in Mafic and Ultramafic Rocks* (eds Buchannan, D. L. & Jones, M. J.) 1-13 (Institution of Mining and Metallurgy, London, 1984).
25. Groves, D. I., Barrett, F. M. & McQueen, K. G. *Can. Miner.* **17**, 319-336 (1979).
26. Ewers, W. E. & Hudson, D. R. *Econ. Geol.* **67**, 1075-1092 (1972).
27. Parker, P. thesis, Univ. Western Australia (1984).
28. Leshner, C. M., Lee, R. F., Groves, D. I., Bickle, M. J. & Donaldson, M. J. *Econ. Geol.* **76**, 1714-1728 (1981).

Estimating the error of age interpolation in sedimentary rocks

Catherine Badgley*, Lisa Tauxe† & Fred L. Bookstein‡

* Museum of Paleontology and ‡ Center for Human Growth and Development, University of Michigan, Ann Arbor, Michigan 48109, USA

† Scripps Institution of Oceanography, La Jolla, California 92093, USA

Magnetostratigraphic data can provide information on rates of sediment accumulation within a single sedimentation system over time spans from 10^4 to 10^6 yr. The short-term rate of sediment deposition varies with time; the apparent average rate over any longer interval also depends on the relative durations of periods of deposition, stasis (non-deposition), and erosion. While the average rate can be used to infer the time of occurrence of an event from its stratigraphical position, the inferred age has an uncertainty deriving from the variability in rate of sediment accumulation over all shorter timescales. We analyse here variability in sediment accumulation rates provided by the magnetostratigraphy of Miocene, Siwalik sediments from Pakistan. For long periods ($>10^6$ yr), sediment accumulation is approximately linear through time. Over short intervals (10^4 - 10^5 yr), however, there is considerable variability. To provide an error term for an absolute age interpolated between boundaries of polarity units, we use a resampling technique similar to the statistician's 'bootstrapping'. We illustrate this approach by estimating a standard error for the interpolated age of a biostratigraphical datum: the first appearance of hipparionine equids in the Siwalik sequence near the town of Khaur. The first appearance of "Hipparion" in the Khaur sequence is 9.22 ± 0.09 Myr.

The assignment of absolute age estimates to sedimentary rocks requires either horizons susceptible to radiometric dating or access to the geomagnetic reversal timescale (GRTS). Fossils may indicate relative chronology in sedimentary rocks but do not convey information about absolute age. Thus, stratigraphical sections usually contain an arbitrary scatter of levels from which age information is available. Ages of all other levels are estimated indirectly by linear interpolation between levels of more accurately known age.

Linear interpolation of an estimated age within a stratigraphical section assumes that the sediment accumulation rate

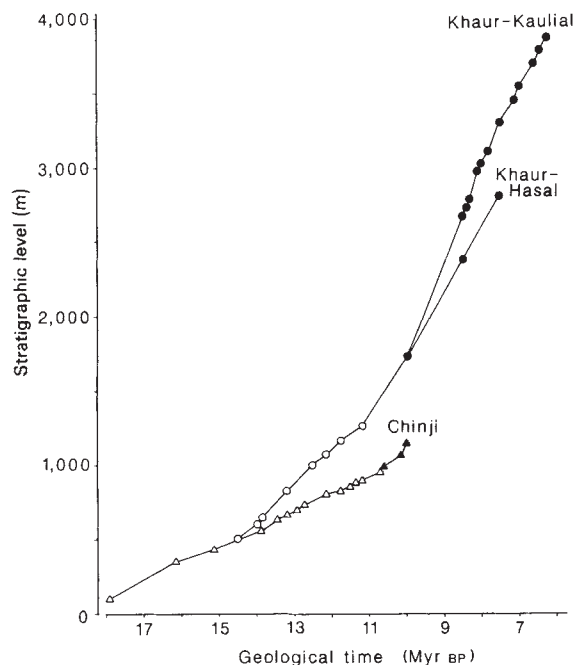


Fig. 1 Cumulative stratigraphic thickness over time in Siwalik sediments of northern Pakistan. Chinji and Khaur are two geographical areas in the same depositional system; Kaulial and Hasal are local section names⁵. Each data point demarcates a reversal of magnetic polarity. Triangles represent data from the Chinji area and circles data from the Khaur area. Solid symbols, strata older than 11 Myr; open symbols, strata younger than 11 Myr. A change in sediment accumulation rate occurred between 10 and 11 Myr, as indicated by the change in slope. Net sediment accumulation appears to be linear through time for the Chinji data older than 11 Myr and for the Khaur data younger than 11 Myr.

calculated for the longer, dated portion of the sequence also applies to much shorter time intervals. This is rarely the case. Unless sediment accumulates continuously and without erosion, sediment accumulation rate decreases as the time span of measurement is increased¹⁻³. Thus it is necessary to consider when linear interpolation of an age estimate is justified, and how to assess the accuracy of the age estimate. Sadler² proposed replacing an interpolated age value by an age range based on an estimated incompleteness of the section. The completeness estimates^{2,4} are based on the pattern of sediment accumulation rate versus time span for a large compilation of data from one class of sedimentary environments, such as fluvial systems. There is, however, no particular reason why any such variance should usefully model the variance within a sedimentation system.

A more direct approach is to exploit observable variability of sediment accumulation rates within a sedimentation system to build a statistical description of the relationship between sediment accumulation and time. The problem in age interpolation is how to estimate the proportion of time span present in a known proportion of sediment thickness from the interval of interpolation. One way to assess the standard error of such ages is to document this variability using intervals of a similar length for which age is not interpolated but is rather observed. Long magnetostratigraphic sections provide independent measurements of sediment accumulation rate for every discernible magnetic polarity interval. We demonstrate a method of 'resampling' a magnetostratigraphic section to generate multiple intervals that can be divided into shorter intervals of known time span and thickness. The original and resampled data together provide an estimate of the standard error about an interpolated age.

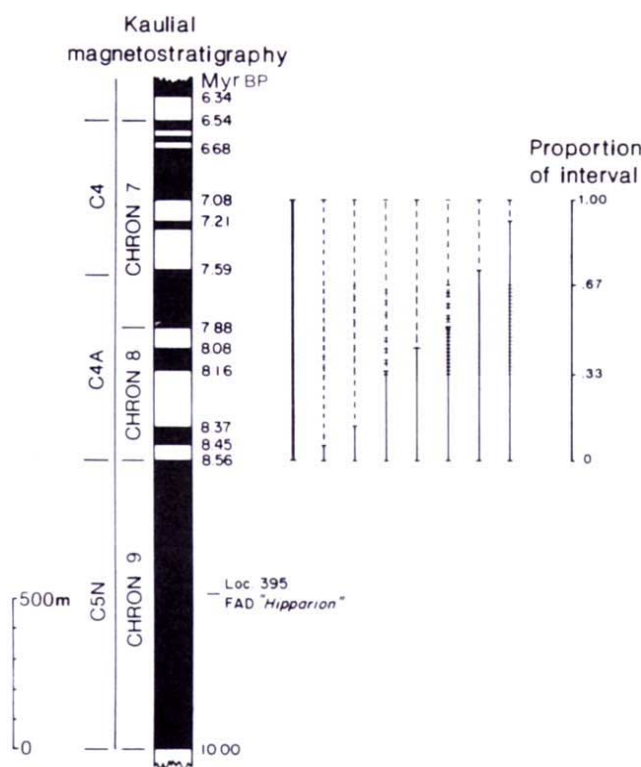


Fig. 2 Magnetostratigraphy from the Kaulial section in the Kaur area⁵ illustrating the resampling method discussed. Absolute ages are indicated from the geomagnetic reversal timescale whenever a polarity interval is represented by at least three palaeomagnetic samples. Two timescales are used: that of Mankinen and Dalrymple⁹ (right) and the new chron names (left) of Berggren *et al.*⁸ given in the text. The first appearance datum (FAD) of "*Hipparion*" occurs within the long normal interval of Chron C5N. On the right, an interval is extracted from the left-hand record that is of about the same duration as Chron C5N. This long interval can be subdivided in seven different ways into complementary segment pairs of known thickness and time span; there result 14 calculations of proportion of sediment thickness and of time span. Of these segment pairs, three divide the long interval into segments between 33 and 67% (stippled band) of the whole. Ten intervals of durations between 1.2 and 1.8 Myr were located in Chrons C4 and C4A; the 104 segment pairs that can be formed within them provide the data in Fig. 3.

The data for demonstrating our approach are contained in two magnetostratigraphic studies of Siwalik sediments from Pakistan^{5,6}. Siwalik rocks are a Neogene molassic sequence representing multiple fluvial systems on the southern margin of the Himalayas⁷. The pattern of Siwalik sediment accumulation through the middle to late Miocene in northern Pakistan is illustrated in Fig. 1. The Kaur sections exhibit higher sediment accumulation rates because they were closer than the Chinji sections to the inferred source area. In both areas, a change in sediment accumulation rate occurred between 10 and 11 Myr in response to an episode of Himalayan uplift⁶. This event, the major secular trend, constitutes a change in the controls of the sedimentation system. In each geographical location, sediment accumulation appears stationary on either side of this event. For the remainder of the discussion, we focus on a portion of the Kaur data.

Magnetostratigraphic data from the Kaur area are documented in 14 measured stratigraphic sections⁵, each spanning part of a 2,100-m interval over a lateral extent of ~25 km. (Only two of these sections are shown in Fig. 1.) The sections are correlated by lithological marker horizons and magnetic polarity stratigraphy; sampling densities ranged from 1 in 3 m to 1 in 23 m. Altogether, the composite Kaur section represents 6.5–

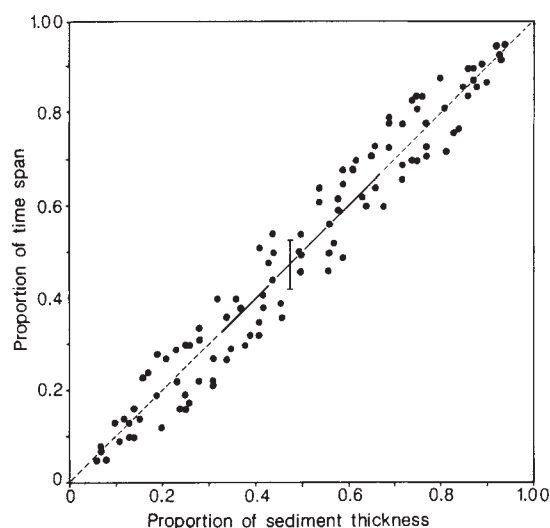


Fig. 3 Variability in proportion of time span in relation to proportion of sediment thickness from subsamples of 10 long intervals in the Kaulial palaeomagnetic section (Fig. 2). Five intervals are longer than the interval of interpolation (1.44 Myr) and five are shorter. Each subsample of a long interval gives rise to two complementary data points whose proportions along either axis sum to 100%. For this reason, the graph is unchanged by a 180° rotation about its centre. The standard error (vertical bar) of 0.06 (6%) was calculated for data in the region of the solid line ($n=40$), corresponding to subsamples of $50 \pm 17\%$ of the whole interval. The value of $\pm 6\%$ appears characteristic of a considerably wider range of sediment proportions, perhaps 18–82%.

10.0 Myr, Chrons C4–C5N (terminology of Berggren *et al.*⁸). (We indicate also the GRTS of Mankinen and Dalrymple⁹ in Fig. 2, for consistency with earlier studies. The choice of time-scale does not affect the results of the technique presented here.) The longest continuous magnetostratigraphic section in deposits younger than 11 Myr, from Kaulial Kas (a local valley name), serves as the basis for the example discussed below (Fig. 2). The sampling density for this section ranged from 1 in 19 m to 1 in 23 m.

Siwalik sediments, rich in mammalian fossil remains, constitute the standard for South Asian, Neogene mammalian biostratigraphy^{10,11}. The first appearance of the three-toed horse "*Hipparion*" in the Kaur Siwalik sequence is significant, both because it marks a rapid faunal turnover within the Siwalik mammalian fauna¹⁰ and because it is used to link Miocene mammalian sites in the Old World¹². "*Hipparion*" includes several hipparionine taxa not always represented by fossils diagnostic to the species level.) Since the first appearance of "*Hipparion*" in the Siwaliks occurs >2 Myr later than its first appearance in European and African sites^{12–15}, this event is also controversial. But it lies in the middle of the long normal interval of Chron C5N, thus being a genuine problem in age interpolation.

In the Kaulial section, the lowest occurrence of "*Hipparion*" is at the Harvard-Geological Survey of Pakistan Locality 395 (J. Barry, personal communication). Stratigraphically, Locality 395 lies 510 m above the base of the long normal unit, which is 940 m thick in Kaulial Kas (Fig. 2)⁵. A linear interpolation estimates this event to be 0.78 Myr younger than the age at the base of the long normal unit, or 9.22 Myr in absolute time. The "*Hipparion*" datum is near the middle of the long normal unit. To assess the expected error of this age estimate, we must know how the proportion of time span varies in relation to about half the sediment thickness for time intervals about the same length as the long normal period. Within the combined sequence of

Chronos C4–C4A, it is possible to delimit various intervals of durations between 1.2 and 1.8 Myr. These intervals can be subdivided into complementary pairs of segments, some representing about half of the full interval. For each segment, the discrepancy between proportion of time span and proportion of sediment thickness is directly observable. Thus, we use the pattern of sediment accumulation for a younger portion of the sediment regime to estimate the variance in an older part of the regime; the implied assumption of stationarity is justified by the linear pattern of cumulative stratigraphic thickness over the entire Kaulial sequence.

The resampling technique is illustrated in Fig. 2. The long interval shown as an example is 1.48 Myr in duration according to palaeomagnetic correlation. This interval contains nine short polarity intervals, of which the smallest, at 7.21 Myr, was represented by less than three samples. It was grouped with the next interval below it. (The same criterion was used to group together the four short intervals from 6.54 to 6.68 Myr in other samples.) The long interval may thus be broken into two pieces of known age in seven different ways. For each division, we calculated the proportion of total sediment thickness and proportion of total time span represented in each segment. In all, 10 long intervals, ranging from 1.25 to 1.77 Myr, were identified and subdivided in a similar fashion: these produced 104 segments, represented in Fig. 3.

For the “*Hipparion*” problem, the variance we need should correspond to a thickness proportion of ~ 0.50 . We computed the mean square

$$\frac{\sum (\text{time proportion} - \text{thickness proportion})^2}{n}$$

of time proportion about thickness proportion for thickness proportions between 0.33 and 0.67 (the solid line in Fig. 3). This quantity is equivalent to the variance in time proportion around a regression line on thickness proportion forced to have a slope of 1 and forced, also, to pass through the origin. For $n = 40$, within the central third of the range, the standard error of this discrepancy is 0.06. Thus, the standard error of the age estimate for the “*Hipparion*” datum should be taken as 6% of 1.44 Myr. By this approach, the age of the first appearance of “*Hipparion*” in the Khaur Siwalik sequence is 9.22 ± 0.09 Myr.

This method of generating a standard error is similar to bootstrapping, jackknifing, and other resampling techniques of modern statistical data analysis. One sample is used to generate many others through intensive resampling of the same data set^{16,17}. The data of Fig. 3 are not statistically independent, even though that was assumed for the data of the original sample. In these circumstances, the computed value of 0.06 is a point estimate only, having no standard error itself.

The value 0.06 pertains to this particular situation, that is, the proportion of time span represented by 33–67% of sediment thickness for intervals of 1.2–1.8 Myr duration in Chrons C4 and C4A in the Kaulial section. The same standard error would apply to an interpolation problem concerning 55% of a polarity interval of 1.7-Myr duration, but not to 55% of an interval of 0.5-Myr duration. For this second case, it would be necessary to generate another sampling distribution. It is ultimately the pattern of the observed palaeomagnetic data which determines how the resampling technique can be applied. It would not be possible, in our example, to determine a standard error for intervals of 0.08 Myr duration, this being the shortest interval represented.

This standard error is not the only source of uncertainty in an interpolated age. Palaeomagnetic dates bear the limitations in time resolution of the GRTS, and the stratigraphic placement of reversal boundaries is partly a function of sample spacing.

These factors contribute additional uncertainty to any palaeomagnetic age estimate (including age interpolations), although the error should not be a systematic one. The standard error calculated by this resampling method can be considered a minimum value attributable to variability in sediment accumulation rate.

Variation in sediment accumulation rates can be attributed to variation in instantaneous sedimentation rates and in completeness of stratigraphic sections. In a complete stratigraphic section with low variability of instantaneous sedimentation rates, linear interpolation provides the best estimate of age. But most stratigraphic sections cannot be considered complete. Even in systems such as lakes or deep ocean basins where deposition is virtually continuous, sediment is reworked by deep currents, turbidite processes, or dissolution. In fluvial systems, such as the Siwaliks, deposition in any one location is markedly discontinuous, even though deposition may occur most of the time somewhere in the system.

In incomplete sections, the results of linear interpolation are most plausible when gaps are relatively short and evenly distributed throughout the sedimentary record. But such convenient models cannot be assumed in general. The distribution of gaps relative to record must be interpreted in part by stratigraphic information from the section(s) under investigation. Fifty meters of laminated silt imply a markedly different distribution of gaps than 50 m of cross-bedded sandstone. When a pattern of sediment accumulation contains long, unevenly distributed discontinuities, linear age interpolation may be very prone to error; and whenever the magnitude of depositional and erosional events varies widely, the record is likely to be dominated by rare events of high magnitude¹⁸.

These considerations argue against the computation of age-estimate errors according to any general stochastic model. In contrast, the resampling technique demonstrated here indicates the root-mean-square amount by which the interpolated age estimate differs from a ‘known’ value as a result of the variability in sediment accumulation rates specific to the particular sequence within which the interpolation is taking place. In this way, each system is characterized by its own pattern of variation in sediment accumulation rates, reflecting its own external factors, such as source-area uplift, or internal factors, such as local reworking of sediment.

We thank Gerald Smith, Jennifer Kitchell and Beth Dawson for constructive discussions. Karen Klitz drew Figs 1 and 2. This work was supported in part by NSF grant EAR 8305931 to C.B. and NSF grant OCE83-17546 to L.T.

Received 5 July; accepted 8 October 1985.

1. Schindel, D. E. *Palaeobiology* **6**, 408–426 (1980).
2. Sadler, P. M. *J. Geol.* **89**, 569–584 (1981).
3. Tipper, J. C. *Nature* **302**, 696–698 (1983).
4. Sadler, P. M. & Dingus, L. W. *Proc. 3rd N. Am. Paleontol. Conv.* **2**, 461–464 (1982).
5. Tauxe, L. & Opdyke, N. D. *Palaeogeogr. Palaeoclimatol., Palaeoecol.* **37**, 43–61 (1982).
6. Johnson, N. M., Stix, J., Tauxe, L., Cervený, P. F. & Tahirkheli, R. A. K. *J. Geol.* **93**, 27–40 (1985).
7. Behrensmeier, A. K. & Tauxe, L. *Sedimentology* **29**, 331–352 (1982).
8. Berggren, W. A., Kent, D. V., Flynn, J. & Van Couvering, J. A. in *Geochronology and the Geological Record* (ed. Snelling, N. J.) (Spec. Pap. Geological Society, London, in the press).
9. Mankinen, E. A. & Dalrymple, G. B. *J. geophys. Res.* **84**, 615–626 (1979).
10. Barry, J. C., Lindsay, E. H. & Jacobs, L. L. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **37**, 95–130 (1982).
11. Barry, J. C., Johnson, N. M., Raza, S. M. & Jacobs, L. L. *Geology* (in the press).
12. Bernor, R. L., Woodburne, M. O. & Van Couvering, J. A. *Geobios* **13**, 705–739 (1980).
13. Ginsburg, L., Janvier, P., Mornand, J. & Pouit, D. C. r. *Somm. Séanc. Soc. Géol. Fr.* **223**–227 (1979).
14. Tiercelin, J.-J., Michaux, J. & Bandet, Y. *Bull. Soc. Géol. Fr.* **21**, 255–258 (1979).
15. Hussain, S. T. & Bernor, R. L. *Himalay. Geol.* **11**, 35–42 (1983).
16. Diaconis, P. & Efron, B. *Scient. Am.* **248** (5), 116–130 (1983).
17. Efron, B. & Tibshirani, R. *Tech. Rep. 101* (Division of Biostatistics, Stanford University, 1985).
18. Crowley, K. D. *J. sedim. Petrol.* **54**, 127–136 (1984).

Light quality and oceanic ultraphytoplankters

Hilary E. Glover, Maureen D. Keller
& Robert R. L. Guillard

Bigelow Laboratory for Ocean Sciences, McKown Point,
West Boothbay Harbor, Maine 04575, USA

Photosynthetic prokaryotic picoplankters ($<2\ \mu\text{m}$), *Synechococcus* spp.^{1,2}, provide a significant fraction of oceanic primary production^{3,4}. The significance of similarly sized eukaryotic algae has not been fully appreciated until recently, as they are difficult to isolate in culture and many become distorted or disintegrate in preservatives^{5,6}. Yet these eukaryotes, in the size range of *Synechococcus*, to perhaps $10\ \mu\text{m}$ (refs 7–10), often numerically dominate ultraphytoplankton ($<10\ \mu\text{m}$) at deep chlorophyll maxima^{5,6,11–13}. Because temporal variability in oceanic productivity seems to be caused by changes at the bottom of the euphotic layer¹⁴, the composition and activity of communities at chlorophyll maxima are of interest. Here we present evidence that eukaryotic ultraplankters have greater photosynthetic and growth efficiencies than *Synechococcus* in the dim blue-violet light occurring at the bottom of the euphotic zone. Our experiments at least partially explain the large numbers of eukaryotic ultraplankters in the open ocean at the 0.5% light level, below the population peak of *Synechococcus* spp.

We compared the performances in culture of oceanic clones of *Synechococcus* and eukaryotic algae from different classes in conditions of nutrient abundance but variable light intensity and quality. We also compared attenuation spectra and fluorescence emission spectra.

All eukaryotic phytoplankters have chlorophyll *a* as their major light-harvesting pigment. Large eukaryotic diatoms and dinoflagellates, which numerically dominate shallow neritic chlorophyll maxima, contain carotenoid pigments (fucoxanthin and peridinin, respectively), which absorb the available green light¹⁵. In contrast, eukaryotic ultraplankton species do not contain these carotenoids but have chlorophyll *b* or *c* as major accessory pigments¹⁶. While oceanic *Synechococcus* spp. contains chlorophyll *a*, phycoerythrin is the major light-harvesting pigment^{17–20}.

Excitation of *Synechococcus* clones with green (530 nm) light produced a much larger fluorescence emission than with blue (470 nm) light. Conversely, ultraplanktonic eukaryotic clones exhibited greater fluorescence emission with blue (470 nm) light than with green (530 nm) light. The ratio of fluorescence emission produced by excitation at 530 nm (green) to that produced by excitation at 470 nm (blue) was calculated for each clone. Except for large diatoms and dinoflagellates, ratios did not exceed 0.7 for eukaryotic algae (0.3–0.4 for ultraplanktonic prasinophytes, 0.4–0.7 for prymnesiophytes and chrysophytes). Highest ratios, 5.2 and 5.4, respectively, were observed for *Synechococcus* clones WH 8018 and WH 7805. These clones contain only phycoerythrobilin (PEB) chromophores in their phycoerythrin¹⁸. *Synechococcus* clones DC-2 and Syn-48 contain phycoerythrin (PUB) in addition to PEB chromophores¹⁸, which shift the excitation wavelength from 530 nm to 510 nm; for these two clones, ratios of fluorescence emission were 2.6 and 2.5 (at 510:470 nm excitation).

We performed photosynthesis and growth experiments in conditions of nutrient abundance but variable light intensity and quality. 'Daylite' fluorescent bulbs in conjunction with coloured filters (Fig. 1) were used to simulate two types of oceanic water²¹—blue-violet (maximum transmittance 455 nm) and blue (maximum transmittance 470 nm)—and coastal water²¹—green (maximum transmittance 542 nm). 'Vitalite' fluorescent bulbs were used to simulate daylight (white light).

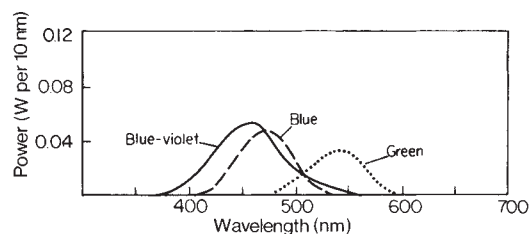


Fig. 1 Spectral quality of plastic filters used in physiological experiments: blue-violet filter (—), maximum transmittance 455 nm; blue filter (---), maximum transmittance 470 nm; green filter (····), maximum transmittance 542 nm.

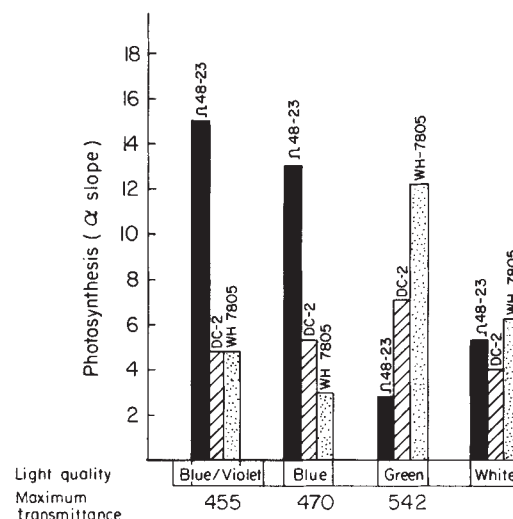


Fig. 2 Photosynthetic efficiencies in different light qualities for an ultraplanktonic prasinophyte clone Ω48-23 (■), *Synechococcus* clone DC-2 (▨) and *Synechococcus* clone WH 7805 (□). Batch cultures were grown at 20 °C, in 14/10 h light/dark cycles in blue-violet light at 1.6×10^{15} quanta $\text{cm}^{-2} \text{s}^{-1}$. In 'log-phase', we determined chlorophyll *a* concentrations²⁴ and photosynthetic rates²⁵ at four rate-limiting fluxes of blue-violet, blue, green or white light, and determined the α slope as a measure of photosynthetic efficiency²². α slope = photosynthesis (mg carbon per mg chlorophyll *a* per h)/irradiance (quanta $\times 10^{-16} \text{ cm}^{-2} \text{s}^{-1}$).

Photosynthetic efficiencies were determined as light-limited α slopes²² in white, green, blue and blue-violet light. All eukaryotic ultraplankton clones used blue-violet light most efficiently for photosynthesis, while phycoerythrin-rich *Synechococcus* clones photosynthesized most efficiently in green light. An example of this is shown in Fig. 2. *Synechococcus* clone DC-2, containing PUB chromophores, was able to use low fluxes of blue light more efficiently for photosynthesis than could *Synechococcus* clone WH 7805, which lacks PUB chromophores. Extending these studies to include growth experiments, we found that eukaryotic ultraplankton clones grew fastest in low fluxes of blue-violet light. In contrast, *Synechococcus* clones grew best in green light (see, for example, Fig. 3).

Even though larger eukaryotic algal clones could effectively use blue-violet light, they could not utilize very low photon flux densities as efficiently as could eukaryotic ultraplankters. This may be partly due to a more efficient absorption of light with decreasing cell size²³. Thus, the bottom of the euphotic zone in the open ocean may provide insufficient photon flux densities for efficient photosynthesis and growth of larger forms.

Our experiments suggest that *Synechococcus* clones containing PUB chromophores should out-compete other *Synechococcus* clones at depth. Moreover, oceanic eukaryotic ultraplankters should have a competitive advantage over *Synechococcus* at the base of the euphotic zone in dim blue-violet light because of

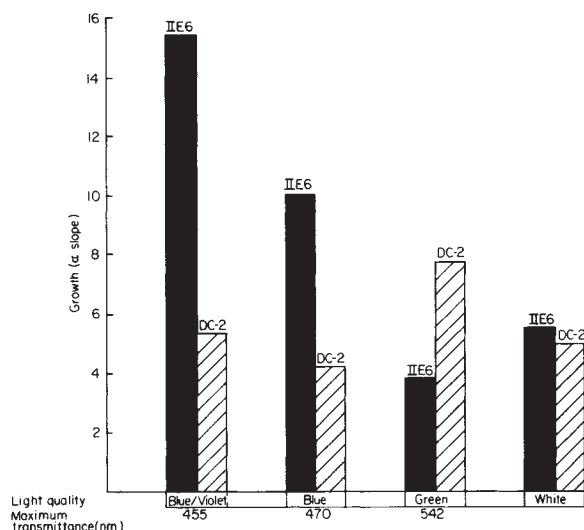


Fig. 3 Growth efficiencies in different light qualities for ultra-planktonic prymnesiophyte clone II E6 (■) and *Synechococcus* clone DC-2 (▨). We determined the daily increase in fluorescence²⁶ and cell numbers in batch cultures at 20 °C, in 14/10 h light/dark cycles at four rate-limiting fluxes of blue-violet, blue, green and white light, and determined the α slope as a measure of growth efficiency. The α slope = specific growth rate (h^{-1})/irradiance ($\text{quanta} \times 10^{-16} \text{ cm}^{-2} \text{ s}^{-1}$).

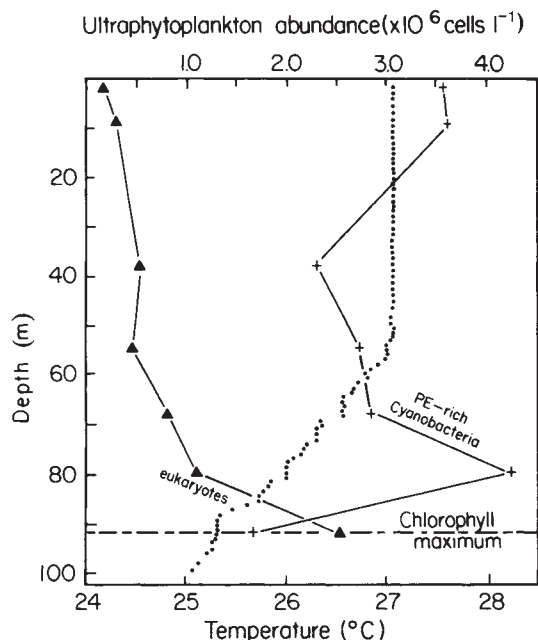


Fig. 4 Depth profiles of phycoerythrin(PE)-rich *Synechococcus* (+), eukaryotic ultraplankton (▲) and temperature (●). Data were collected from the Sargasso Sea at 34°49' N, 66°20.6' W in August 1983. Pump water was immediately filtered at <125 mm Hg through a 3- μm Nuclepore filter. The filtrate was passed through a 0.2- μm Nuclepore filter and cells were classified as either *Synechococcus* or eukaryotes⁶.

functional differences in their pigment compositions. Recent field experiments support this hypothesis²⁰. Ultraplankton sink at negligible rates¹², thus biomass peaks below the surface mixed layer^{6,13} in the open ocean may be attributed to *in situ* growth. One advantage of inhabiting this dimly illuminated environment is the proximity to richer nutrient levels from upward advection across the thermocline. This enrichment enables cells to synthesize additional pigments for photoadaptation. Below the upper isothermal layer in the Sargasso Sea (Fig. 4), we found the

maximum numbers of *Synechococcus* occurring at about 1% of surface irradiance, while the maximum abundance of eukaryotic ultraplankton occurred below this, constituting a chlorophyll maximum. This agrees with previous data from the central gyre of the North Atlantic⁶.

It should also be noted that photosynthetic studies in deck-incubators, under natural light modulated in intensity but not quality, will underestimate the contribution of eukaryotic ultraplankton to the *in situ* rate of oceanic primary production.

We thank E. M. Haugen, A. E. Smith and R. Spinard for assistance with this research and C. S. Yentsch and L. A. Codispoti for reviewing this manuscript. The work was supported by NSF grants OCE-84-05299 and OCE-82-08626. This is Bigelow contribution 84034.

Received 30 April; accepted 24 December 1985.

1. Waterbury, J. B., Watson, S. W., Guillard, R. R. L. & Brand, L. E. *Nature* **277**, 293–294 (1979).
2. Johnson, P. W. & McN. Sieburth, J. *Limnol. Oceanogr.* **24**, 928–935 (1979).
3. Li, W. K. W. *et al. Science* **219**, 292–295 (1983).
4. Platt, T., Subba-Rao, D. V. & Irwin, B. *Nature* **301**, 702–704 (1983).
5. Furuya, K. & Marumo, R. *J. Plankton Res.* **5**, 393–406 (1983).
6. Murphy, L. S. & Haugen, E. M. *Limnol. Oceanogr.* **30**, 47–58 (1985).
7. Thronsen, J. *Sarsia* **63**, 273–284 (1978).
8. Thronsen, J. *Norw. J. Bot.* **23**, 269–293 (1976).
9. Johnson, P. W. & McN. Sieburth, J. *J. Phycol.* **8**, 318–327 (1982).
10. Yentsch, C. S. *Limnol. Oceanogr.* **28**, 597–599 (1983).
11. Takahashi, M. & Hori, T. *Mar. Biol.* **79**, 177–186 (1984).
12. Takahashi, M. & Bienfang, P. K. *Mar. Biol.* **76**, 203–211 (1983).
13. Glover, H. E., Smith, A. E. & Shapiro, L. *J. Plankton Res.* **7**, 519–535 (1985).
14. Bienfang, P. K., Szyper, J. P., Okamoto, M. Y. & Noda, E. K. *Limnol. Oceanogr.* **29**, 527–539 (1984).
15. Yentsch, C. S. & Yentsch, C. M. *J. mar. Res.* **37**, 471–483 (1979).
16. Foss, P., Guillard, R. R. L. & Liaaen-Jensen, S. *Phytochemistry* **23**, 1629–1633 (1984).
17. Kursar, T. A., Swift, H. & Alberte, R. S. *Proc. natn. Acad. Sci. U.S.A.* **11**, 6888–6892 (1981).
18. Alberte, R. S., Wood, A. M., Kusar, T. A. & Guillard, R. R. L. *Pl. Physiol.* **75**, 732–739 (1984).
19. Ong, L. J., Glazer, A. N. & Waterbury, J. B. *Science* **224**, 80–83 (1984).
20. Wood, A. M. *Nature* **316**, 253–255 (1985).
21. Jerlov, N. G. *Optical Oceanography*, 127 (Elsevier, Amsterdam, 1968).
22. Platt, T. & Gallegos, C. L. in *Primary Productivity in the Sea* (ed. Falkowski, P. G.) 339 (Plenum, New York, 1980).
23. Morel, A. & Bricaud, A. *Deep-Sea Res.* **11**, 1375–1393 (1981).
24. Yentsch, C. S. & Menzel, D. W. *Deep-Sea Res.* **10**, 443–448 (1963).
25. Morris, I. & Glover, H. E. *Mar. Biol.* **24**, 147–154 (1974).
26. Brand, L. E., Guillard, R. R. L. & Murphy, L. S. *J. Plankton Res.* **3**, 193–207 (1981).

Birds that 'cry wolf'

Charles A. Munn

Wildlife Conservation International, New York Zoological Society,
The Bronx Zoo, Bronx, New York 10460, USA

Reports of animals using alarm calls deceptively are rare (refs 1–3 and R. Cheney and D. Seyfarth, personal communication in ref. 4). Here I have studied two species of flycatching birds in Amazonia, *Lanio versicolor* and *Thamnomanes schistogynus*, which lead flocks of mixed species in the canopy and understorey of the forest, respectively, and act as sentinels, giving alarm calls at the approach of bird-eating hawks. These two species feed to a large extent on the insects flushed out by the foraging of the rest of the flock. My observations suggest that *L. versicolor* and *T. schistogynus* use the predator alarm call deceptively to distract other birds, thereby increasing their own chances of capturing arthropods. This result suggests that deception among animals may be more widespread than is generally assumed.

The mixed-species bird flocks of the Amazon forest are the most diverse multi-species associations described for any organisms^{5–8}. At Cocha Cashu, Manu National Park, south-east Peru (71° 19' W, 11° 51' S, elevation ~400 m), the white-winged shrike-tanager (*L. versicolor*, Thraupinae) leads permanent mixed-species flocks in the canopy of the forest and helps maintain flock cohesion by means of loud vocalizations throughout the day^{6–8}. At the same site, the bluish-slate antshrike (*T. schistogynus*, Formicariidae) performs the same role in permanent mixed-species flocks in the understorey^{5–8}.

In addition to their roles as flock leaders, these two species are also flock sentinels, as they are virtually always the first

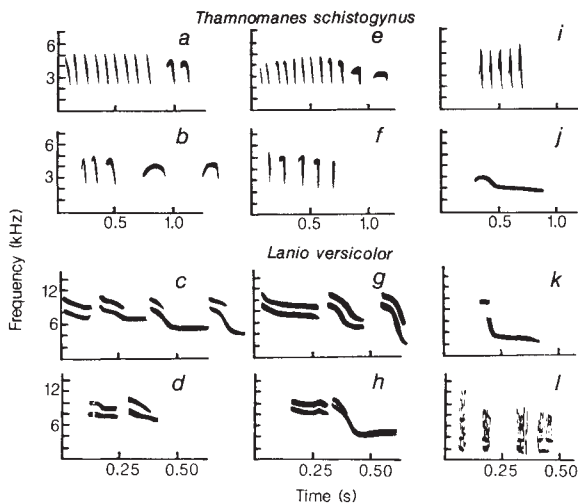


Fig. 1 Tracings of spectrograms of true alarm calls (a–d), false alarm calls (e–h), and other (non-alarm) calls (i–l) of *T. schistogynus* and *L. versicolor*. True alarms are given in response to real danger; false during a chase after arthropods; and other calls in entirely different contexts. The non-alarm rattle (i) was used as a control in the experiment. True and false alarms of *T. schistogynus* are characterized by repeated staccato notes of narrower bandwidth than the notes of the non-alarm rattle (i), which apparently is given to promote flock cohesion^{4–6}. The true and false alarms of *L. versicolor* are characterized by descending notes with simple, strong harmonic structure; both characteristics are not found in other calls. Note that for *L. versicolor*, the timespan is halved, while the frequency range is doubled compared with the results for *T. schistogynus*. Spectrograms were made on a Kay Elemetrics 6061B machine with wide-band filter.

species to give loud alarm calls at the approach of bird-eating hawks of the genera *Micrastur*, *Accipiter* and *Leucopternis*^{5,6,8}; they are also the first to give short alarm calls when harmless hawk-sized birds fly by⁶. Finally, they are also the first species to sound an alarm when hawk models are shot past them⁶. Other species of flock birds look up, freeze, dive or jump into cover when a sentinel sounds the alarm.

The sentinel role has enabled *T. schistogynus* and *L. versicolor* to establish a novel symbiotic relationship with other flock members. Both sentinel species rely on the insect-flushing abilities of other flock species for $\geq 85\%$ of their food⁶. Rarely do they steal arthropods directly from the bills of other birds. Rather, they sit in the centre or beneath a group of actively foraging flock species and fly out or dive down after dropping arthropods flushed from hiding by the more active species. When a bird of another species begins to chase an arthropod that it has flushed out, the faster-flying, more aerobic sentinel often catches the arthropod first. It is during these multi-bird aerial tumbles after arthropods that both species of sentinel give the same alarm call used when a hawk attacks or flies by. I interpret these calls as false alarm calls, presumably used by the sentinel to distract other birds and thereby to increase its own chance of capturing the arthropod. These aerial contests are over in less than a second, so even a slight hesitation by other birds increases the likelihood that the sentinel will reach the arthropod first.

Using the following criteria, I classified 106 of 718 alarm calls as true or false: true if simultaneously I saw a hawk-like object fly by or if other flock species subsequently alarmed and froze for several minutes, and false if the sentinel flew into the open after a falling arthropod while I simultaneously had a clear view of the entire region within 20 m of the bird and could thus eliminate the possibility of a passing hawk. Sentinels remained motionless on partially concealed perches when giving true alarms, whereas when giving false alarms, they flew with other birds into the open in pursuit of flushed arthropods. Flying into

Table 1 Response of other understory flock species to playback of true and false alarms and rattle (control) of *T. schistogynus*

	Bird reacts	Bird does not react
Rattle (control) (i in Fig. 1)	0	15
True alarm call (a in Fig. 1)	13	0
False alarm call (e in Fig. 1)	13	3
		$P > 0.153$
		$P < 0.001$

Other birds are equally startled by the true and false alarms of *T. schistogynus*, while they are not startled by the rattle. Fisher exact tests were used to calculate the probabilities associated with the two comparisons. Recordings and playback were via a Panasonic RQ 356 cassette recorder, JVC shotgun microphone, and a modified Olympus SMW SP5 field speaker.

the open is inappropriate for escape from fast-flying bird hawks. Furthermore, the probability is vanishingly small that hawk-like birds repeatedly flew by at the same instant that sentinels were competing with other birds for falling arthropods, as neither type of incident occurred more than three times per hour.

The context of the false alarms further strengthens my interpretation. *L. versicolor* sounded the alarm significantly less often when it alone was chasing an arthropod than when another bird was involved also (19 instances out of 131 versus 34 out of 56, $\chi^2 = 39.0$, $P < 0.001$). Furthermore, the rate of sounding of alarms increased dramatically when either sentinel species was feeding its young. As most of these additional alarms were given during multi-bird chases after flushed arthropods, and the young were often too far from the adults to hear the calls, I classified most of these additional alarms as false. Thus, the rate of sounding of alarm calls was higher when the opportunity for obtaining more food was greater and when the need for that food was more pressing.

Known true and false alarms are strikingly similar in outline and frequency on spectrograms, whereas both true and false alarms are very different from other calls in the repertoire of the two sentinel species (Fig. 1). Although alarm calls can vary from one or two notes to a whole series of sharp notes, only the first one or two are needed to trigger a startle or escape reaction from individuals of other species.

The postulated effect of the alarm calls, indeed the effect of only hearing the calls, was confirmed by an experiment in which I played back cassette recordings of a non-alarm rattle call, a known true alarm, and a known false alarm of *T. schistogynus* to understory flock species. The birds treated both the true and the false alarms as true alarms, while they ignored the rattle (control) (Table 1). The canopy flocks foraged too high to permit the same experiment, but observation of natural true and false alarms of *L. versicolor* supported the interpretation of deceptive alarm calls. Even though about half of all *L. versicolor* alarm calls are false (56 out of 104 categorizable alarm calls), canopy flock birds reacted positively to 31 of 41 alarm calls of unknown context, which is significantly greater than the percentage of true alarms (46%; $\chi^2 = 9.14$, $P < 0.01$). On two of these 31 occasions, I saw another bird react to the call as if it were a true alarm call and then saw the *Lanio* fly out after an insect. In the 10 cases in which a bird showed no reaction to a sentinel alarm call, the bird was already in the open and was thus able to scan for hawks without moving. In contrast, birds with their heads inside leaves, or under their wings, bathing, or in other vulnerable positions, typically showed the most dramatic escape responses by diving for cover when any alarm call was given.

Theoretically, as in the story of the boy who cried "Wolf!" (ref. 9), there should be an upper limit to the proportion of false alarms that can be supported by a given rate of occurrence of true alarms. The potential penalty, however, for ignoring even

one alarm call might be death; so it is not surprising that flock birds seem to take all alarm calls seriously, whether true or false. Presumably, the cost to the beater species of reacting to false alarms is the occasional loss of an arthropod to the sentinel, while the only cost to the sentinel is the energy used in producing the call. The possible benefit to the sentinel of sounding a false alarm could be a substantial increase in its daily intake of arthropods. Unfortunately, the outcomes of most of the multi-bird scrambles for arthropods were concealed by vegetation, so I cannot compare the rate of prey capture by sentinels when silent with their capture rates when giving false alarms.

In general, an animal competing with others for a resource might use deceptive alarm calls in contests of predictably short duration in which rivals who check for danger automatically lose.

I thank the Frank M. Chapman Fund of the American Museum of Natural History for support; W. J. Smith for use of his sonograph machine; J. Gould, D. Griffin, H. Horn and F. Nottebohm for constructive criticism; and the Ministry of Agriculture of Peru for permission to work in Manu National Park.

Received 29 May; accepted 5 November 1985.

1. Ruppell, G. Z. *Tierpsychol.* **26**, 371-374 (1969).
2. Wilson, E. O. *The Insect Societies* (Harvard University Press, 1971).
3. Matsuoka, S. *Tori* **29**, 87-90 (1980).
4. Dennett, D. *The Behavioural and Brain Sciences* Vol. 6, 343-355 (1983).
5. Munn, C. A. & Terborgh, J. W. *Condor* **81**, 338-347 (1979).
6. Munn, C. A. thesis, Princeton Univ. (1984).
7. Munn, C. A. in *Neotropical Ornithology* (eds Buckley, P. A., Foster, M. S., Morton, E. S., Ridgely, R. S. & Buckley, F. G.) (American Ornithological Union, Washington DC, 1985).
8. Wiley, R. H. *J. Zool.* **191**, 127-145 (1980).
9. Davis, R. H. *Boy Scout and Other Stories for Boys* (Scribner's, New York, 1917).

Synapsin I is a microtubule-bundling protein

Anthony J. Baines* & Vann Bennett

Department of Cell Biology and Anatomy,
Johns Hopkins University School of Medicine,
725 North Wolfe Street, Baltimore, Maryland 21205, USA

Synapsin I, a synaptic vesicle protein, is thought to be involved in the regulation of neurotransmission through its phosphorylation by the cyclic AMP-dependent and Ca^{2+} /calmodulin-dependent protein kinases which become activated upon depolarization of nerve endings¹⁻³. However, despite its recent characterization⁴ as a spectrin-binding protein immunologically related to erythrocyte protein 4.1, other interactions of synapsin I with structural proteins remain unknown. We report here that synapsin I can co-cycle with microtubules through three cycles of warm polymerization and cold depolymerization. Synapsin I binds saturably to microtubules stabilized by taxol, with an estimated dissociation constant (K_d) of $4.5 \mu\text{M}$ and a stoichiometry of 1.2 mol of synapsin binding sites per mol tubulin dimer. Synapsin I also increases the turbidity of tubulin solutions at 37°C , but without causing detectable alterations in the critical concentration required for polymerization. Mixtures of synapsin I and tubulin observed by negative stain electron microscopy contain bundles of microtubules, accounting for the effect of synapsin I on tubulin turbidity. Synapsin I is thus a candidate to mediate or regulate the interaction of synaptic vesicles with microtubules.

Synapsin I was prepared from calf brains, as described in Fig. 1, by modifications of our previous procedure⁴. Tubulin was prepared from three-times-cycled microtubule protein from calf brain by chromatography on Whatman DE53 cellulose⁵. In

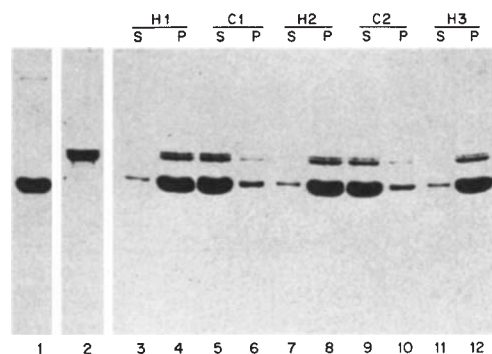


Fig. 1 Synapsin I co-cycles with tubulin during three cycles of warm polymerization and cold depolymerization. Supernatants (S) and pellets (P) from the first, second and third warm centrifugation steps (H1, H2 and H3), as well as those for cold centrifugation (C1 and C2) were analysed on an SDS-polyacrylamide gel. Lane 1, DEAE-purified tubulin (30 μg); 2, synapsin I (15 μg); 3, H1S; 4, H1P; 5, C1S; 6, C1P; 7, H2S; 8, H2P; 9, C2S; 10, C2P; 11, H3S; 12, H3P.

Methods. Synapsin was purified by modifications of our previous procedure⁴. Briefly, synapsin I was extracted from demyelinated brain membranes in the presence of 0.15 M NaCl, 1% Triton X-100 pH 7.1, and purified by chromatography on hydroxylapatite and carboxymethyl cellulose (A.J.B. and V.B., manuscript in preparation). Synapsins 1a and b were more than 90% of the protein in the final preparation, with a further 6-8% being proteolytic degradation products of relative molecular mass (M_r) $> 50,000$ (see lane 2, above). Tubulin was purified from three-times-cycled microtubules by DEAE chromatography⁵. Tubulin was about 98% of the final preparation, the major contaminant being MAP-1, which constituted no more than 1% of the total protein (see gel lane 1, above). For the experiments shown in Figs 2, 3 and 4, the tubulin was completely freed of MAP-1 by centrifugation of microtubules in the presence of 0.5 M NaCl: the microtubule pellet formed under these conditions contains no detectable MAP-1. Tubulin was stored as a microtubule pellet at -80°C , and was cycled once prior to use. In the cycling experiment shown, synapsin I (7 μM) was combined with tubulin (66 μM) at 0°C in PMGDE buffer (see text for buffer composition). Glycerol was added to give a final concentration of 20% (v/v) and the mixture was brought to 37°C for 30 min. The microtubules that formed during the incubation were collected by centrifugation for 25 min at 150,000g, and were depolymerized in one-third of the initial volume of ice-cold PMGDE. After incubation for 30 min on ice, with intermittent shearing through a 26-gauge needle, the mixture was clarified by centrifugation at 150,000g for 25 min at 0°C . Glycerol was added to the supernatant after this spin, the mixture brought to 37°C , and the cycle repeated twice more. Samples were withdrawn from all supernatants and resuspended pellets produced for SDS-polyacrylamide gel electrophoresis. The pellets from C1 and C2 were resuspended in 1% SDS. Concentrations of synapsin I were estimated spectrophotometrically, assuming $E_{1\text{cm}}^{277} = 6.74$ at 277 nm (ref. 12). Synapsin was assumed to be a monomer of M_r 75,000. Tubulin concentrations were estimated by a dye-binding assay²⁵ using bovine serum albumin as standard: molar concentrations were calculated assuming a tubulin dimer of M_r 100,000.

preliminary experiments, synapsin I (0.5-5 μM) co-sedimented with microtubules after incubation with tubulin (10-50 μM , with reference to dimer) at 37°C for 30 min, in a buffer optimal for microtubule assembly (0.1 M Na-PIPES, 1 mM MgCl_2 , 1 mM GTP, 1 mM dithiothreitol (DTT), 1 mM EGTA, 1 mM $\text{Na}_2\text{S}_2\text{O}_8$, pH 6.6: PMGDE buffer) (data not shown). However, an interaction between synapsin I and tubulin dimer was not detectable by sedimentation of mixtures of synapsin I and tubulin through linear sucrose gradients at 4°C , suggesting that synapsin I will interact strongly only with tubulin in polymeric form.

One characteristic of many microtubule-associated proteins (MAPs) is that they co-cycle with tubulin during repeated cycles of warm polymerization and cold depolymerization. When mixtures of synapsin I and tubulin were subjected to repeated

* Present address: University of Kent, Canterbury, UK.

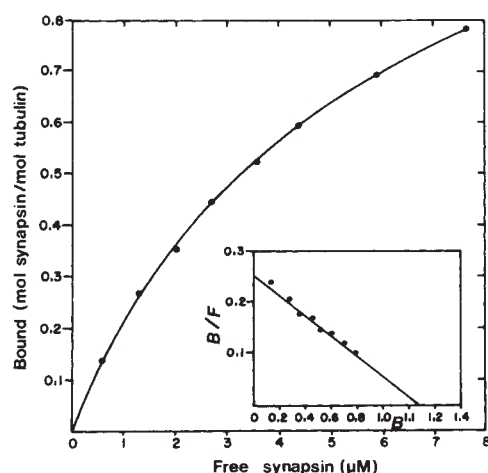


Fig. 2 Binding of ¹²⁵I-synapsin I to taxol-stabilized microtubules. *a*, Association of various concentrations of ¹²⁵I-synapsin I with 1.0 μM taxol-tubulin. Binding is expressed as mol synapsin I bound per mol tubulin (dimer), after subtraction of blank values. *b*, Data plotted according to the Scatchard equation, $B/F = N/K - B/K$ (ref. 9), where B is moles of synapsin bound per mole of tubulin (dimer), F is the concentration of unbound synapsin (μM), N is the number of binding sites (mol mol⁻¹) and K is the dissociation constant. The line shown is a calculated best fit for a single class of sites, which yields $K = 5$ μM and $N = 1.2$.

Methods. Synapsin I was labelled with ¹²⁵I-labelled Bolton Hunter reagent and diluted with unlabelled synapsin I to give 8.76×10^5 c.p.m. nmol⁻¹. DEAE-purified tubulin (52 μM dimer) was polymerized in PMGDE buffer without glycerol, and taxol was added from a 10 mM solution at 37 °C for 20 min. The resulting microtubules were resistant to cold depolymerization, and >98% of the tubulin was pelletable during centrifugation at 100,000g for 25 min. In the binding assay, taxol-tubulin was diluted to a final concentration of 1.0 μM (relative to dimer), with various concentrations of ¹²⁵I-synapsin I in PMGDE buffer, to give a volume of 100 μl, and allowed to stand for 1 h at 23 °C. Samples of 90 μl were layered over 100 μl of 20% sucrose, 20 μM taxol in PMGDE, in Beckman airfuge tubes. Taxol-stabilized microtubules, with bound ¹²⁵I-synapsin, were pelleted during centrifugation at 100,000g for 30 min in the airfuge. Aliquots of 50 μl supernatant were withdrawn from the meniscus, and the remaining supernatant aspirated. Supernatants and pellets were counted for ¹²⁵I in a γ-counter. Blanks, from which tubulin was omitted, were included in the assay. All points were determined in duplicate, the means of which are shown.

cycles of warm polymerization and cold depolymerization, synapsin I co-cycled with microtubules (Fig. 1). (Synapsin I alone showed no tendency to polymerize in the warm.) The ratio of synapsin I to tubulin in the experiment shown decreased by only 5% during three cycles (as judged by elution of dye from Coomassie blue-stained SDS-polyacrylamide gels of the pellets, followed by reading absorbance at 605 nm)⁶, and we conclude that co-cycling reflects a stable interaction between tubulin polymer and synapsin I.

To quantitate such an interaction, the association of ¹²⁵I-synapsin with taxol-stabilized microtubules was measured. Taxol is an antimetabolic drug⁷ which virtually eliminates the critical concentration required for tubulin polymerization⁸, allowing the use of very dilute tubulin polymers for saturation binding analysis. Binding of 0–7.5 μM ¹²⁵I-synapsin was examined at both 1.0 μM (Fig. 2) and 1.2 μM (not shown) tubulin (concentrations being expressed relative to dimer). When the data were plotted according to the Scatchard equation⁹, a single class of binding sites (1.2 mol binding sites per mol tubulin dimer) was revealed, with a K_d of 4.5 μM. Experimental limitations on the concentration of synapsin prevented more than about 60% saturation of tubulin; thus additional lower-affinity classes of binding sites or negative coopera-

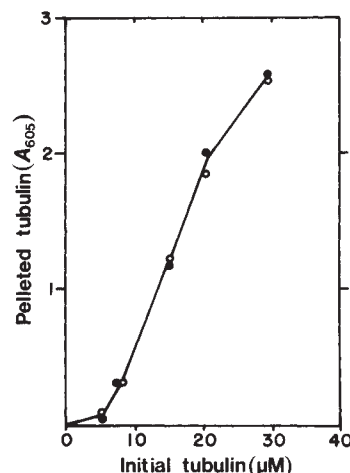


Fig. 3 Synapsin I does not alter the critical concentration for polymerization of tubulin. Various concentrations of tubulin (4.9–29.4 μM) were incubated with (●) or without (○) 5 μM synapsin I in PMGDE buffer containing 5% glycerol. After 30 min at 37 °C, the mixtures were centrifuged for 25 min at 100,000g, and samples of supernatants and pellets analysed by gel electrophoresis: tubulin was quantitated by dye elution from Coomassie-stained gels⁶. Data are plotted as total tubulin (μM) against tubulin pelleted (expressed as A_{605} of dye eluted from stained gels). The critical concentration is assumed to be represented by the point at which the linear portion of the curve intercepts with the x-axis. A critical concentration of 4–5 μM tubulin is estimated in the presence and absence of synapsin I.

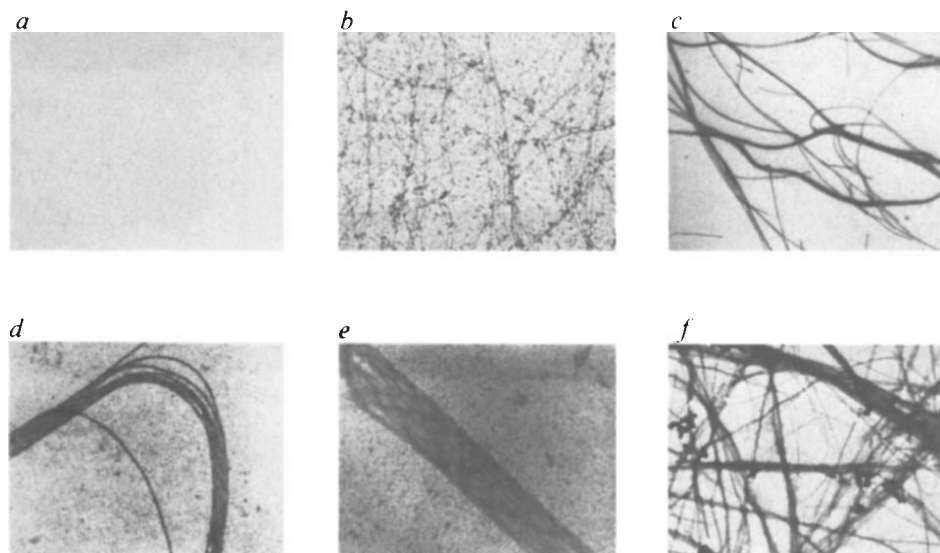
tivity may become apparent at higher concentrations of synapsin (4.5 μM may be a minimum estimate for the K_d as drug-treated tubulin may not be as active as native tubulin). Synapsin I may also undergo limited self-association in the assay conditions used (see below), which may affect the apparent stoichiometry of the reaction.

Synapsin I increases the turbidity of tubulin solutions when incubated at 37 °C (not shown). Microtubule-associated proteins also do this, seemingly by reducing the critical concentration required for polymerization (see for instance refs 10, 11). That synapsin I might act similarly was examined in Fig. 3. Various concentrations of tubulin were incubated in PMGDE buffer containing 5% glycerol, in the presence or absence of 5 μM synapsin I. After 30 min at 37 °C, microtubules were collected by centrifugation. Pelleted tubulin was separated from bound synapsin by electrophoresis on SDS-polyacrylamide gels, and quantitated by dye elution from Coomassie blue-stained gels⁶. Synapsin I had no detectable effect on the final extent of tubulin polymerization, and a critical concentration of 4–5 μM is estimated both in the presence and absence of synapsin I (Fig. 3). By contrast, the addition of MAP-2 to tubulin in our assay conditions greatly reduces the critical concentration.

Since the increase in turbidity of tubulin solutions could not be accounted for by lowering of the critical concentration, synapsin I/tubulin mixtures were examined by negative stain electron microscopy (Fig. 4). While control tubulin formed single microtubules of normal appearance, randomly arranged on electron microscope grids, mixtures of synapsin I and tubulin revealed bundles of microtubules in tightly packed arrays. The extent of bundling was dependent on the amounts of synapsin I and tubulin in the incubation mixtures: at high concentrations of synapsin I and tubulin (for example 5 μM and 25 μM respectively), the bundles were sufficiently large to be easily distinguished in the light microscope.

Cross-linking of microtubules by synapsin implies that either synapsin has two or more binding sites for microtubules, or that synapsin has one tubulin binding site and achieves cross-linking by self-association. Synapsin is monomeric in dilute solution^{4,12}; however, in concentrated solutions of synapsin (1.5 mg ml⁻¹),

Fig. 4 Electron microscopy of synapsin I, tubulin and synapsin I/tubulin mixtures. Synapsin I ($5 \mu\text{M}$) and tubulin ($10 \mu\text{M}$ or $25 \mu\text{M}$) were incubated separately or together at 37°C in PMGDE buffer in the absence of glycerol. After 30 min, samples were applied to Formvar carbon films on 400-mesh grids. The samples were rinsed with buffer, stained with 1% unbuffered uranyl acetate and examined in the electron microscope. *a*, Synapsin I alone; *b*, tubulin ($10 \mu\text{M}$) alone; *c*, *d*, *e*, synapsin I plus tubulin ($10 \mu\text{M}$); *f*, synapsin I plus tubulin ($25 \mu\text{M}$). The scale bar (lower right) represents $4.7 \mu\text{m}$ in *a*, *b*, *c* and *f*, $1.2 \mu\text{m}$ in *d* and $0.6 \mu\text{m}$ in *e*.



oligomers of synapsin up to tetramer in size are revealed by HPLC gel filtration or by chemical cross-linking (A.J.B. and V.B., unpublished data, but see also ref. 12). Therefore, it is possible that bundling of microtubules is dependent on self-association of synapsin into multivalent complexes. In future work it will be important to investigate the factors that regulate self-association, and the self-association state of synapsin in its vesicle-bound form.

In spite of repeated reports, based on electron microscopy, that synaptic vesicles might be attached to microtubules¹³⁻¹⁶, the cytoskeletal interactions of synaptic vesicles are still very unclear (compare refs 14 and 17 but see also ref. 18). However, interactions between secretory vesicles and microtubules have been detected in reconstitution experiments^{19,20} and, more recently, in taxol-stabilized complexes of microtubules and secretory granules isolated from exocrine pancreas²¹. Our observations suggest that synapsin I might act to link or regulate the linkage of microtubules to synaptic vesicles. The role of such a linkage is open to speculation, but it might play a part in axonal transport of vesicles, or in the placement of synaptic vesicles in the presynaptic active zones. The association of synaptic vesicles with microtubules is likely to be transient *in vivo*, as it is known that the interaction of synapsin I with its membrane binding site is weakened upon phosphorylation by Ca^{2+} /calmodulin-dependent protein kinase². Furthermore, in view of alterations in the activities of MAP-2^{22,23} and tau²⁴ in response to their phosphorylation, it is possible that the tubulin binding activity of synapsin will be altered by its phosphorylation. It is not yet known how the spectrin-binding activity of synapsin I relates to its tubulin-binding activity.

This work was supported by a Damon Runyon-Walter Winchell Cancer Fund Fellowship FRG700 and by NIH grants AM29808, RO 1 GM33996 and RCDA KO4 AM00926. We thank Arlene Daniel for preparation of the manuscript.

10. Murphy, D. B., Vallee, R. B. & Borisy, G. G. *Biochemistry* **16**, 2598-2605 (1977).
11. Kim, H., Binder, L. I. & Rosenbaum, J. L. *J. Cell Biol.* **80**, 266-276 (1979).
12. Ueda, T. & Greengard, P. *J. Biol. Chem.* **252**, 5155-5163 (1977).
13. Jarlfors, U. & Smith, D. S. *Nature* **224**, 710-711 (1969).
14. Smith, D. S. *Phil. Trans. R. Soc. B* **261**, 395-405 (1971).
15. Bird, M. M. *Cell Tissue Res.* **168**, 101-115 (1976).
16. Gray, E. G. *Proc. R. Soc. B* **218**, 253-258 (1983).
17. Wickelgren, W. O., Leonard, J. P., Grimes, M. J. & Clark, R. D. *J. Neurosci.* **5**, 1188-1201 (1985).
18. Llinas, R. R. & Heuser, J. E. *Neurosci. Res. Prog. Bull.* **15**, 557-687 (1977).
19. Sherline, P., Lee, Y. C. & Jacobs, L. S. *J. Cell Biol.* **72**, 380-389 (1977).
20. Suprenant, K. A. & Dentler, W. L. *J. Cell Biol.* **93**, 164-174 (1982).
21. Dentler, W. L. & Suprenant, K. L. *J. Cell Biol.* **99**, 193a (1984).
22. Murthy, A. S. N. & Flavin, M. *Eur. J. Biochem.* **137**, 37-46 (1983).
23. Burns, R. G., Islam, K. & Chapman, R. *Eur. J. Biochem.* **141**, 609-615 (1984).
24. Lindwall, G. & Cole, R. D. *J. Biol. Chem.* **259**, 5301-5306 (1984).
25. Flores, R. *Analyt. Biochem.* **88**, 605-611 (1978).

Functional corticotropin releasing factor receptors in the primate peripheral sympathetic nervous system

Robert Udelsman*, James P. Harwood, Monica A. Millan, George P. Chrousos†, David S. Goldstein‡, Reuven Zimlichman‡, Kevin J. Catt & Greti Aguilera

Endocrinology and Reproduction Research Branch, NICHD; * Surgery Branch, NCI; † Developmental Endocrinology Branch, NICHD; and ‡ Hypertension-Endocrine Branch, NHLBI, National Institutes of Health, Bethesda, Maryland 20892, USA

Corticotropin releasing factor (CRF) is a key hormone in the integrated response to stress, acting both as the major regulator of pituitary adrenocorticotrophic hormone (ACTH) release and as a neuropeptide in the brain^{1,2}. The actions of CRF are mediated by specific plasma membrane receptors in the anterior pituitary gland³ and in discrete brain areas including the cerebral cortex and several regions related to the limbic system^{4,5}. In addition to the pituitary and central actions of CRF, systemic administration of the peptide in the rat⁶, dog^{7,8}, monkey⁹ and man¹⁰⁻¹² causes hypotension and tachycardia because of a decrease in peripheral vascular resistance. These observations, in conjunction with the finding of immunoreactive¹³⁻¹⁷ and bioactive^{14,18} CRF in peripheral tissues, suggest that the peptide is locally released in tissues to act as a neurotransmitter or paracrine hormone. As CRF is present in the adrenal medulla^{13,14} and the peptide is known to modulate

Received 19 June; accepted 14 November 1985.

1. Nestler, E. J. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7479-7483 (1980); *Nature* **296**, 452-454 (1982).
2. Hutner, W. B., Schiebler, W., Greengard, P. & DeCamilli, P. *J. Cell Biol.* **96**, 1374-1388 (1983).
3. Llinas, R., McGuinness, T. L., Sugimori, M. & Greengard, P. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3035-3039 (1985).
4. Baines, A. J. & Bennett, V. *Nature* **315**, 410-413 (1985).
5. Murphy, D. B. *Meth. Cell Biol.* **24**, 31-49 (1982).
6. Fenner, C., Traut, R. R., Mason, D. T. & Wikman-Coffelt, J. *Analyt. Biochem.* **63**, 603-606 (1975).
7. Wani, M. C., Taylor, H. L., Hall, M. E., Coggon, P. & McPhail, A. T. *J. Am. chem. Soc.* **93**, 2325-2327 (1971).
8. Schiff, P. B., Fant, J. & Horwitz, S. B. *Nature* **277**, 665-667 (1979).
9. Scatchard, G. *Ann. N.Y. Acad. Sci.* **57**, 660-672 (1949).

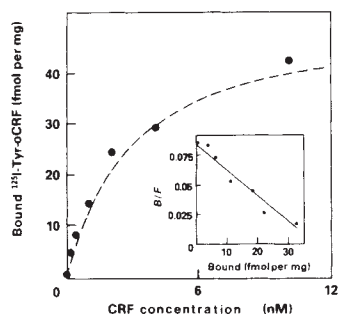


Fig. 1 Saturation curve and Scatchard analysis (inset) of ^{125}I -Tyr-ovine CRF binding to adrenal medulla membrane particles in the rhesus monkey. Each point represents the mean of duplicate incubations from a representative experiment.

Methods. Tissue was minced and homogenized in a 50 mM-Tris-HCl buffer (pH 7.4) containing 5 mM MgCl_2 and 2 mM EGTA, by six strokes on a mechanical homogenizer (Techmar). Homogenates were filtered through nylon gauze and centrifuged at 30,000g for 30 min at 5 °C. The membrane-rich pellet was resuspended in the same buffer to give a protein concentration of 2–8 mg ml^{-1} . Aliquots of 100 μl of membrane suspension were incubated with 100,000 c.p.m. of ^{125}I -Tyr-ovine CRF (0.1 nM) in a total volume of 300 μl of 50 mM Tris-HCl buffer containing 5 mM MgCl_2 , 2 mM EGTA, 0.1% bovine serum albumin (BSA) and 100 kIU ml^{-1} of aprotinin (Sigma). After incubation for 30 min at 22 °C, bound radioactivity was separated from free tracer by centrifugation at 1,000g for 3 min at 4 °C following addition of 7.5% polyethylene glycol (PEG) in 50 mM Tris-HCl, pH 7.4. The precipitate was washed again with PEG, and the tips of the tubes were removed and analysed for radioactivity in a Beckman γ -spectrometer. Tracer degradation after 60 min incubation was <10% as evaluated by rebinding to rat pituitary membranes. Receptor concentration and affinity were estimated by computer analysis of the binding data³⁵, and protein concentration was measured³⁶ with BSA as a standard.

the central activity of the autonomic nervous system¹, we investigated the possibility that CRF is involved in the regulation of the peripheral autonomic nervous system. Such an action of CRF is supported by our demonstration of specific CRF receptors in the monkey adrenal medulla and sympathetic ganglia. In the adrenal medulla, these receptors are coupled to adenylate cyclase and can stimulate the secretion of catecholamines and Met-enkephalin.

Adrenal glands and lumbar, coeliac, thoracic and cervical sympathetic ganglia were obtained from cynomolgus and rhesus monkeys within 5 min of killing them with an overdose of pentobarbital. The adrenals were cleaned of connective tissue and dissected into three zones; medulla, zona glomerulosa and the inner zones of the cortex. CRF receptors were measured by binding of ^{125}I -Tyr-ovine CRF to the 30,000g membrane-rich fractions of tissue homogenates as described previously³. No CRF binding was found in either zona glomerulosa or inner zones of the cortex, but saturable, high-affinity binding was present in the adrenal medulla (Fig. 1). Specific binding of CRF in the adrenal medulla was linear up to 800 μg of membrane protein and reached equilibrium between 30 and 60 min at 22 °C with a half-maximum association time of 16 min. Addition of unlabelled oCRF at equilibrium resulted in rapid dissociation of the bound radioactivity with a half-time of 25 min. Scatchard analysis of the binding data revealed a single class of receptors with dissociation constant (K_d) of 5×10^{-9} and 2.3×10^{-9} M in cynomolgus and rhesus monkeys, respectively. The mean value of three experiments including both species was $3.2 \pm 1.0 \times 10^{-9}$ M. The binding capacity was also similar in both species, with values of 47 fmol mg^{-1} for cynomolgus and 39.2 ± 4.2 fmol mg^{-1} for the rhesus. This binding capacity of the adrenal medulla membrane fraction was similar to that observed in rat brain⁴ and much less than that found in the rat pituitary gland².

The ligand specificity of the medullary binding sites for ^{125}I -Tyr-oCRF is shown in Fig. 2. The native peptides oCRF and human CRF were equipotent, with half-maximum inhibitory

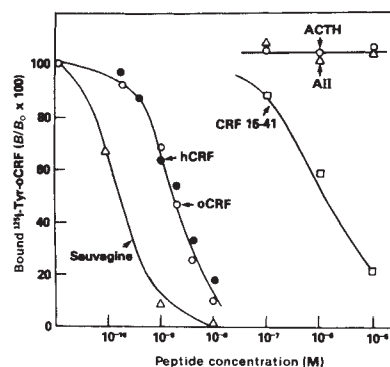


Fig. 2 Ligand specificity of medullary CRF sites in the rhesus monkey. Each point is the mean of duplicate incubations in a representative experiment, performed by the addition of increasing concentrations of CRF and other peptides to adrenal medulla particles incubated with ^{125}I -Tyr-ovine CRF.

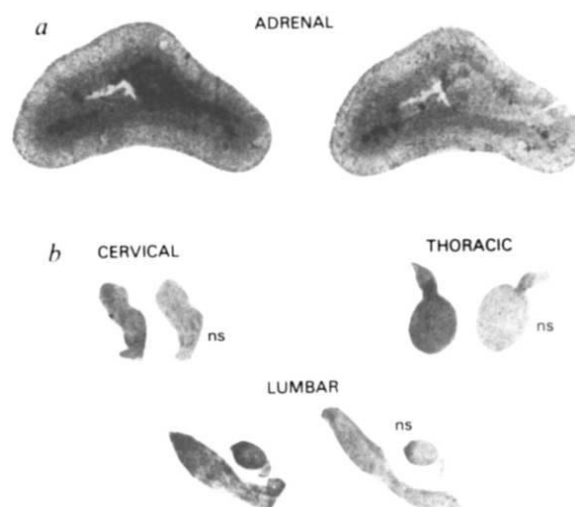


Fig. 3 Autoradiographic analysis of ^{125}I -Tyr-ovine CRF binding to 20- μm frozen sections of monkey adrenal (a) and sympathetic ganglia (b). Slide-mounted sections were incubated for 1 h at 22 °C with 0.2 nM ^{125}I -Tyr-ovine CRF in the absence and in the presence of 1 μM unlabelled oCRF (for nonspecific binding), then washed four times in CRF-free incubation buffer, dried and exposed in X-ray cassettes to LKB ultrafilm for 6 days at room temperature^{4,37}.

concentrations (IC_{50}) of 1.95 ± 0.1 nM and 2.1 ± 0.2 nM, respectively. The structurally related peptide, sauvagine⁸, was more potent than CRF, whereas the CRF(15–41) fragment was 1,000 times less potent with an IC_{50} of 1.1 μM . The structurally unrelated peptides, ACTH and angiotensin II, showed no binding-inhibition activity at concentrations up to 10^{-5} M. This specificity is similar to that described for CRF receptors in the anterior pituitary gland³ and in the brain⁴. Similarly, the relative affinities of CRF-related peptides to the adrenal receptor are consistent with the potencies of these peptides to stimulate ACTH release *in vivo*^{1,19} and *in vitro*^{20,21}, and to decrease blood pressure following intravenous administration^{6–9}.

Binding sites for ^{125}I -Tyr-oCRF in the adrenal medulla were also evident on autoradiographic analysis of the binding in slide-mounted frozen sections of rhesus monkey adrenal glands. Consistent with the results in adrenal membranes, CRF receptors were confined to the adrenal medulla, with no specific autoradiographic staining in the adrenal cortex (Fig. 3a). Autoradiographic analysis also demonstrated specific CRF binding in several sympathetic ganglia including lumbar, thoracic, cervical (Fig. 3b), and coeliac (not shown). Analysis of the autoradiograms by computerized densitometry indicate optical densities of 0.69 ± 0.05 and 0.23 ± 0.03 , respectively, in the

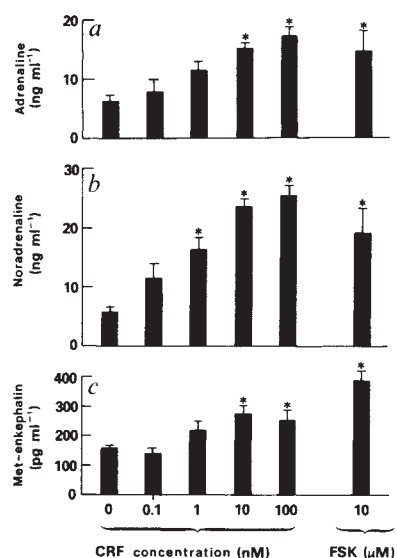


Fig. 4 Adrenaline, noradrenaline and Met-enkephalin production by bovine chromaffin cells during 24 h incubation with corticotropin releasing factor (CRF) or forskolin (FSK). Each bar indicates the mean $\pm$ s.e. from triplicate incubations. Asterisks indicate significant differences ($P < 0.05$) as determined by Duncan's multiple range test³⁸.

Methods. Chromaffin cells were isolated from bovine adrenal glands after retrograde adrenal vein perfusion and collagenase treatment³⁹ and cultured in 24-well plates (Falcon) containing 1.5 ml per well of basal Eagle's medium with Earle's salts and 25 mM HEPES buffer (Gibco), 5% heat inactivated fetal calf serum, glutamine, penicillin and streptomycin. After incubation for 3 days at 37 °C the media was replaced and either CRF or FSK was added. After an additional 24 h incubation in the presence of CRF or FSK the media were acidified with 150 μ l of 1 N HCl and frozen for subsequent assays. Catecholamine levels were determined by HPLC with electrochemical detection⁴⁰. Met-enkephalin levels were determined by radioimmunoassay⁴¹ after ODS-silica column extraction (ImmunoNuclear Corp.).

absence or presence of excess unlabelled CRF. In all sympathetic ganglia, complete inhibition of the specific binding was observed with 10 nM CRF, indicating the high affinity of the binding sites.

As the actions of CRF in well-recognized target tissues such as the pituitary^{1,21} and brain⁴ are accompanied by stimulation of adenylate cyclase, we determined whether CRF binding sites are associated with a similar function in the adrenal medulla by examining the ability of the peptide to stimulate the conversion of ³²P-ATP to cyclic AMP²¹. CRF caused a small but significant and dose-dependent stimulation of adenylate cyclase activity in the adrenal medulla. In three experiments, incubation of membrane-rich particles with ovine CRF increased the enzyme activity by $31 \pm 5\%$, from a basal value of 35.2 ± 1.8 pmol mg⁻¹ min⁻¹ to a maximum of 46.2 ± 3.2 pmol mg⁻¹ min⁻¹, with an ED₅₀ of $7.2 \pm 1.9 \times 10^{-8}$ M CRF. Stimulation of adenylate cyclase was consistently observed with nanomolar concentrations of CRF, although the ED₅₀ for enzyme activation was higher than the CRF receptor affinity, as frequently observed in broken cell preparations^{4,21-24}. The relatively small degree of stimulation over basal probably reflects the multiplicity of cell types in the adrenal medulla, of which only a small proportion contain CRF receptors.

To determine whether activation of adrenal medullary CRF receptors causes changes in secretory activity, the effect of CRF on catecholamine release was analysed in cultured bovine chromaffin cells. During short-term incubation (10–30 min), CRF had no effect on catecholamine secretion, whereas nicotine caused a 30-fold stimulation over the basal value for both adrenaline and noradrenaline. However, after culture of the chromaffin cells in the presence of CRF for 24 h there was a significant increase ($P < 0.05$) in catecholamine release, to a

maximum of threefold of the basal values for adrenaline (Fig. 4a) and fourfold for noradrenaline (Fig. 4b) with 10 mM CRF. A further increase in catecholamine release was observed at longer times of culture with 10 nM CRF (eightfold at 48 h and tenfold at 72 h), whereas nicotine-stimulated values remained at a plateau (5-fold). After incubation with nicotine for 72 h, the level of adrenaline increased from 5 ± 1 to 25 ± 1 ng ml⁻¹ and that of noradrenaline from 7.1 to 37 ± 3 ng ml⁻¹. A much greater stimulation to 52 ± 1 ng ml⁻¹ adrenaline and 69 ± 1 ng ml⁻¹ for noradrenaline was observed after incubation with CRF for the same period. The concentrations of CRF required to stimulate catecholamine release were consistent with the K_d of the CRF receptor in the monkey adrenal medulla.

Several peptide hormones have been associated with the function of the adrenal medulla. Vasoactive intestinal peptide (VIP), somatostatin, and many of the proopiomelanocortin derivatives including enkephalin, β -endorphin, ACTH and α -MSH have been identified by radioimmunoassay in normal adrenal medulla and pheochromocytomas^{15,25-28}. Several of these peptides are concomitantly released with catecholamines during stimulation of chromaffin cells with carbachol, nicotine or high potassium, suggesting that they are also involved in the stress response²⁵⁻²⁷. Studies with cultured bovine chromaffin cells have shown stimulation of VIP and Met-enkephalin synthesis and release, and an increase in proenkephalin messenger RNA in cultures exposed to high levels of cyclic AMP or forskolin^{30,31}.

The ability of CRF to stimulate adenylate cyclase activity in the adrenal medulla suggested that the peptide could also modulate the secretion of enkephalin in chromaffin cells. As shown in Fig. 4c, addition of CRF to cultured bovine chromaffin cells for 24 h caused a significant increase in Met-enkephalin release, to a maximum of twice the basal value ($P < 0.05$) with 10 nM CRF. Forskolin, a known stimulator of adenylate cyclase³², also enhanced Met-enkephalin and catecholamine release.

The properties of the binding sites for CRF in the adrenal medulla, with specificity for CRF-related peptides, high affinity and the ability to stimulate adenylate cyclase and the secretion of catecholamines and Met-enkephalin, indicate that they represent functional receptors through which CRF modulates adrenal medullary function. The presence of similar binding sites in sympathetic ganglia suggests that CRF is also involved in the extra-adrenal peripheral autonomic nervous system. Both CRF and its receptors are present in the central nervous system at sites where the peptide is known to modify the activity of the autonomic nervous system. The CRF that is present in normal adrenal medulla and pheochromocytomas¹³⁻¹⁵ could be locally released to serve as a paracrine or autocrine regulator of the secretion of other stress hormones. Such cyclic AMP-mediated effects of CRF could be particularly relevant during prolonged activation of the medulla, as suggested by the latency of the effect of CRF on catecholamine release, and may be important in long-term adaptation to stress.

A still unresolved question is the functional interaction between the adrenal medulla and cortex. As the circulation within the adrenal gland appears to flow from the cortex to the medulla^{33,34}, it is difficult to propose a mechanism by which peptides and/or catecholamines released from the medulla could have a direct effect in the cortical zones. However, it is possible that secretory products of the medulla can reach the cortex via local circulatory shunts or simple diffusion. Although the physiological role of CRF in the peripheral autonomic nervous system will require further study, the presence of the peptide and its functional receptors in the adrenal medulla and sympathetic ganglia suggest that CRF regulates the activity of the peripheral autonomic nervous system. These findings are consistent with the notion that CRF has a major role in the integrated response of the organism to stress, with actions at all levels of the neuroendocrine effector system.

We thank Robin W. Stull, Charles Grewe and Thomas P. Tomai for technical assistance.

Received 3 June; accepted 14 November 1985.

1. Vale, W., Spiess, J., Rivier, C. & Rivier, J. *Science* **213**, 1394–1397 (1981).
2. Aguilera, G. *et al. J. Neuroendocr.* (in the press).
3. Wynn, P. C., Aguilera, G., Morell, J. & Catt, K. J. *Biochem. biophys. Res. Commun.* **110**, 602–609 (1983).
4. Wynn, P. C. *et al. Peptides* **5**, 1077–1084 (1984).
5. De Souza, E. B. *et al. Science* **224**, 1449–1450 (1984).
6. Fisher, L. A., Brown, M. R. in *Current Topics in Neuroendocrinology* (eds Ganten, D. & Pfaff, D.) 87 (Springer, Berlin, 1983).
7. MacCannell, K. L., Lederis, K., Hamilton, P. L. & Rivier, J. *Pharmacology* **25**, 116–120 (1982).
8. Brown, M. R. *et al. Regulat. Peptides* **4**, 107–114 (1982).
9. Udelman, R. *et al. in The Adrenal Gland and Hypertension* (eds Mantero, F. & Biglieri, E.) (Raven, New York, in the press).
10. Orth, D. N. *et al. J. clin. Invest.* **71**, 587–595 (1983).
11. Hermus, A. *et al. Lancet* **i**, 776 (1983).
12. Schürmeyer, T. H. *et al. J. clin. endocr. Metab.* **59**, 1103–1108 (1984).
13. Suda, T. *et al. J. clin. endocr. Metab.* **58**, 919–924 (1984).
14. Hashimoto, K. *et al. Peptides* **5**, 707–711 (1984).
15. De Cherney, G. S., Nicholson, W. E., Jackson, R. V. & Orth, D. N. *Clin. Res.* **32**, 264A (1984).
16. Petrusz, P., Merchenthaler, I., Maderdrut, J. L., Vigh, S. & Schally, A. V. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1721–1725 (1983).
17. Nieuwenhuijzen Kruseman, A. C., Linton, E. A., Lowry, P. J., Rees, L. H. & Besser, G. M. *Lancet* **ii**, 1245–1246 (1982).
18. Carey, R. M. *et al. New Engl. J. Med.* **311**, 13–20 (1984).
19. Schürmeyer, T. H. *et al. Endocrinology* **117**, 300–302 (1985).
20. Vale, W. *et al. Endocrinology* **113**, 1121–1131 (1983).
21. Aguilera, G. *et al. J. biol. Chem.* **258**, 8039–8045 (1983).
22. Mukherjee, C., Caron, M. C. & Lefkowitz, R. J. *Endocrinology* **99**, 347–357 (1976).
23. Lin, M. C., Lin, C.-S. & Whitlock, J. P. *J. biol. Chem.* **254**, 4684–4688 (1979).
24. Harwood, J. P., Reichert, N. D., Dufau, M. L. & Catt, K. J. *Endocrinology* **106**, 280–288 (1980).
25. Corder, R. *et al. Neuropeptides* **3**, 9–17 (1982).
26. Eiden, L. E., Eskay, R. L., Scott, J., Pollard, H. & Hotchkiss, A. J. *Life Sci.* **33**, 687–693 (1983).
27. Evans, C. J., Erdelyi, E., Weber, E. & Barchas, J. D. *Science* **221**, 957–960 (1983).
28. Berenyi, M. R., Singh, G., Gloster, E. S., Davidson, M. I., Woldenberg, D. H. *Archs Path.* **101**, 31–35 (1977).
29. Wilson, S. P., Chang, K. J. & Viveros, O. H. *J. Neurosci.* **2**, 1150–1156 (1982).
30. Eiden, L. E. & Hotchkiss, A. J. *Neuropeptides* **4**, 1–9 (1983).
31. Quack, T. T. *et al. Molec. Pharmacol.* **26**, 255–260 (1984).
32. Seamon, K. B., Padgett, W. & Daly, J. W. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3363–3367 (1981).
33. Sjöstrand, T. *Skand. Archs Physiol.* **71**, 85–122 (1934).
34. Hamaji, M. & Harrison, T. S. in *Blood Vessels and Lymphatics in Organ Systems* (eds Abramson, D. I. & Dobrin, P. B.) 280–295 (Academic, New York, 1984).
35. Ketelslegers, J. M., Knott, G. D. & Catt, K. J. *Biochemistry* **14**, 3075–3083 (1975).
36. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
37. Quirion, R., Hammer, R. P., Herkenman, M. & Pert, C. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5581–5585 (1981).
38. Duncan, D. B. *Biometrics* **11**, 1–42 (1955).
39. Greenberg, A. & Zinder, O. *Cell Tissue Res.* **226**, 655–656 (1982).
40. Goldstein, D. S., Feuerstein, G., Izzo, J. L. Jr, Kopin, I. J. & Keiser, H. R. *Life Sci.* **28**, 467–475 (1981).
41. Miller, R. J., Chang, K. J., Cooper, B. & Cuatrecasas, P. *J. biol. Chem.* **253**, 531–538 (1978).

IgE-mediated degranulation of mast cells does not require opening of ion channels

M. Lindau* & J. M. Fernandez

Department of Membrane Biophysics, Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen, FRG

Rat peritoneal mast cells respond to antigenic stimulation by releasing histamine through exocytosis. The dynamics of exocytosis can be investigated by dialysing single cells with patch pipettes using the whole-cell recording configuration of the patch-clamp technique^{1,2}. However, dialysed cells fail to respond to external stimuli such as compound 48/80 or antigens, suggesting that essential cytoplasmic components have been washed out. We have developed a new patch-clamp configuration in which the patch under the pipette tip is not disrupted but instead permeabilized, preventing the diffusion of large molecules out of the cell. In this configuration the cell responds to external stimulation, and the capacitance as well as the conductance of the cell membrane can be recorded during degranulation. On antigenic stimulation, the cell capacitance (proportional to plasma membrane area), after an initial delay, increases by a factor of about 3. This increase in capacitance is often preceded by a transient increase in conductance. Agents that block Ca-activated channels inhibit this conductance change without affecting the amplitude and time course of degranulation. We therefore conclude that, in contrast to excitable secretory cells such as chromaffin cells², mast cells do not use ion channels in stimulus-secretion coupling.

When antigens were added to cells passively sensitized with immunoglobulin E (IgE), the cell being dialysed with the patch pipette did not respond and no inward currents were detected (less than 1 pA at +5 mV), whereas the surrounding cells degranulated within a few minutes (Fig. 1). This suggests that essential components had been washed out or inactivated by the pipette solution (Fig. 2a), breaking the chain between stimulus and exocytosis. The idea underlying the present work was to make the interior of the cell accessible not by disrupting the patch under the pipette by means of suction, but by permeabilizing it so as to create small pores. This would prevent the rapid diffusion of large molecules out of the cell (Fig. 2b) so that it would remain responsive to external stimuli.

Mast cells are permeabilized if the extracellular side of the plasma membrane is exposed to ATP³ (ref. 3). When a pipette containing ATP, no calcium and a calcium chelator (EGTA or bis(O-aminophenoxy)ethane N,N,N',N'-tetraacetic acid, BAPTA) was sealed against the surface of a mast cell, the membrane patch under the pipette became permeable and a capacitive current transient could be measured in response to a voltage pulse (Fig. 1c, inset). As in the whole-cell configuration, the membrane capacitance (C_m) and conductance (G_m) of the cell as well as the access resistance (R_a) were derived from this transient. Typical values obtained were 200–5,000 MΩ for the patch access resistance (corresponding to ~200 Ω cm²) and a plasma membrane resistance (R_m) of 20–100 GΩ (~20,000 Ω cm²). The values for R_m and C_m (5–10 pF) agree very closely with the corresponding results obtained in the whole-cell configuration^{1,4}. In a few cells the patch was disrupted, and measurements confirmed the previously obtained values. As the main difference between the standard whole-cell mode⁵ and our method is the time constant of the capacitive transient, we propose the name 'slow whole-cell' ($C_m R_a \sim 10$ ms) for this new method, as opposed to the 'fast whole-cell' ($C_m R_a \sim 100$ μs) obtained by patch disruption.

In the slow whole-cell configuration, mast cells degranulate together with the neighbouring cells in response to extracellular stimuli such as compound 48/80 or multivalent antigens. Figure 1c shows a group of mast cells that had been passively sensitized with IgE. Recordings were made from one cell of the group in the slow whole-cell mode (capacitive transient shown in the inset). On stimulation with dinitrophenyl linked to bovine serum albumin (DNP₄₃-BSA), all the mast cells degranulated simultaneously (Fig. 1d). The capacitive transient did not change in amplitude but became about three times slower, indicating that the capacitance of the cell had increased about threefold without a significant change in access resistance. A small increase in membrane conductance also occurred after degranulation (increased steady-state current in inset of Fig. 1d). The result shown in Fig. 1c,d supports the idea of a wash-out of essential cellular components by the fast whole-cell method (Fig. 2a).

Permeabilization of the patch provides a new method for monitoring exocytosis in undisturbed, intact mast cells. We anticipate that this method will be applicable to other cell types by the use of pore-forming substances, thus enabling the monitoring of events that require an intact cytosol. The slow whole-cell method is similar to microelectrode recording but, unlike the latter technique, causes little damage to the cells.

Figure 3a shows typical time courses of changes in the cell membrane capacitance and conductance following stimulation with DNP₄₃-BSA. In the slow whole-cell mode, capacitance

* Present address: Biophysics Group, Department of Physics, Freie Universität Berlin, D-1000 Berlin 33, FRG.

Fig. 1 Typical dispersions of cells from a rat peritoneal lavage. Mast cells are easily recognized by their large nucleus and granular appearance. The cells shown here were passively sensitized with monoclonal IgE before the experiment. *a*, A single mast cell in this group has been dialysed with our standard intracellular solution. Dialysis is accomplished in the whole-cell mode of the patch-clamp technique. In this configuration, a -20 -mV voltage pulse (from 0 mV) induces a transient current (inset, calibration bars 1 ms, 2 nA), which corresponds to the charging of the cell membrane capacitance. The steady-state current is negligible due to the absence of ionic conductances in the mast cell membrane. *b*, On addition of DNP_{43} -BSA to the bath, all the mast cells except for the dialysed cell degranulate. The capacitive transient is also unmodified, implying that the membrane area, membrane conductance and pipette access resistance have not changed. Dialysis of rat peritoneal mast cells in these conditions thus renders the cells refractory to external stimulation, which suggests the loss of cytoplasmic components essential for stimulus-secretion coupling. *c*, A patch pipette containing a solution including 400 μM ATP and 400 μM BAPTA is sealed against one mast cell of the group. The membrane patch is permeabilized by this solution, allowing electrical measurements of the cell capacitance and conductance. The inset shows the current transient in response to a -20 -mV pulse (inset, calibration bars 100 ms, 10 pA). This transient is similar to the one obtained in the fast whole-cell configuration (*a*), but is about 100 times smaller and slower, as expected from the large access resistance R_a (see Fig. 2*c*). The resting cell parameters as determined from this transient are: $R_a = 1.9$ G Ω , $C_m = 6.3$ pF, $R_m = 34$ G Ω (29 pS). *d*, The same group of cells 5 min after addition of DNP_{43} -BSA to the bath. The cells, including the one in slow whole-cell mode, degranulate simultaneously. The time constant of the capacitive transient (inset) is increased about threefold, but the amplitude is the same as in *c*, indicating an increase in the cell membrane capacitance by a factor of ~ 3 , as expected from a degranulated cell. The steady-state current at the end of the pulse is also larger than before stimulation, indicating that the membrane conductance increased slightly. Cell parameters: $R_a = 1.9$ G Ω , $C_m = 18$ pF, $R_m = 7$ G Ω (140 pS).

Methods. The cells were incubated for more than 1 h with monoclonal IgE (1 $\mu\text{g ml}^{-1}$) at 37°C before use. The chamber with the cells was mounted in the patch-clamp set-up and perfused with extracellular saline to wash off the unbound IgE. The external solution was then exchanged for a medium containing 145 mM NaCl, 2.8 mM KCl, 1 mM MgCl_2 , 2 mM CaCl_2 , 10 mM HEPES, 25 mM glucose, 100 μM phosphatidylserine. The pipette solution contained 150 mM K-glutamate, 10 mM HEPES, 7 mM MgCl_2 , 200 μM Na_2ATP , 200 μM BAPTA. All solutions were adjusted to pH 7.2 with NaOH. A 20 - μl aliquot of a solution of DNP_{43} -BSA in phosphate-buffered saline (100 $\mu\text{g ml}^{-1}$) was added in the vicinity of the observed cells to stimulate degranulation.

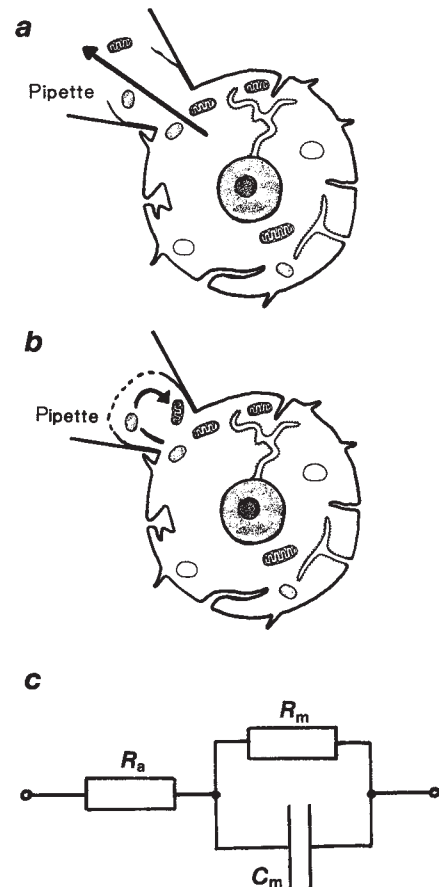
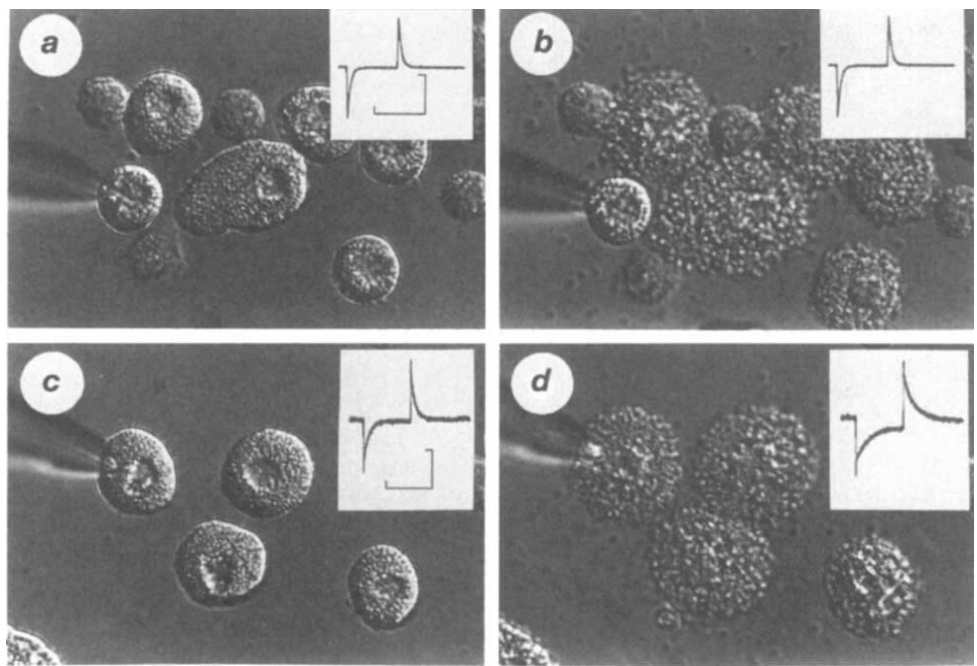
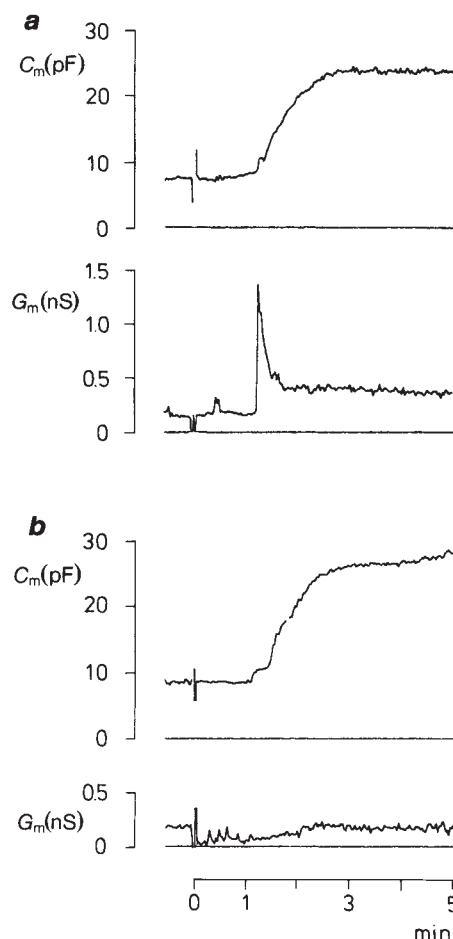


Fig. 2 Schematic representation of the fast and slow whole-cell configurations. *a*, Fast whole-cell configuration. The patch under the pipette has been disrupted by suction after seal formation. The diameter of the tip can be larger than 2 μm , allowing the diffusion of cytoplasmic components out of the cell volume (<1 pL) into the large volume of the pipette (~ 25 μl). *b*, Slow whole-cell configuration. The patch under the pipette is not disrupted but permeabilized, preventing the rapid loss of large cytoplasmic molecules and allowing the permeation of small ions. *c*, Equivalent circuit for both whole-cell configurations. The seal resistance between the interior of the pipette and the bath has been neglected. If a voltage pulse is applied, a capacitive transient is evoked which can be fitted by a single exponential. From the current response, C_m , R_m and R_a can be calculated. For mast cells, typical values are 5 – 10 pF for C_m and 20 – 100 G Ω for R_m , corresponding to a whole-cell conductance of 10 – 50 pS for a resting cell. The range of access resistance (R_a) is 1 – 20 M Ω in the fast whole-cell mode (*a*) and 100 – $5,000$ M Ω in the slow whole-cell mode (*b*). If the current transient is recorded both in the slow whole-cell configuration and after patch disruption in the fast whole-cell mode, it is possible to determine the seal conductance. In these experiments the seal conductance was usually less than 15 pS.

Fig. 3 Time course of capacitance and conductance of the mast cell membrane during IgE-mediated degranulation, recorded in the slow whole-cell configuration. *a*, Typical degranulation under normal conditions. Extracellular medium as in Fig. 1. DNP₄₃-BSA was added to the bath at time zero, causing the artefacts in the traces. The capacitance increased by a factor of ~3 after an initial delay of ~80 s (upper trace). The capacitance increase was preceded by a conductance transient of ~1.3 nS (lower trace). It probably corresponds to the opening of Ca-activated ion channels induced by a transient rise in the intracellular free calcium concentration. However, such transients were not observed in all cells. *b*, Degranulation in the presence of 10 μ M pimozone (a gift from Janssen Pharmaceuticals). External solution as in Fig. 1, but containing 10 mM CaCl₂ and 10 μ M pimozone. Pimozone was added from a 10 mM stock solution in ethanol. The time course and amplitude of the capacitance increase (upper trace) were similar to those shown in *a*. However, no significant conductance change was observed before degranulation. The addition of DNP₄₃ was accompanied by a conductance decrease from 180 to ~50 pS in this experiment, probably due to an accidental improvement in the seal resistance. The following slow conductance increase is superimposable on the capacitance change, suggesting that the conductance increase simply reflects the increase of membrane area with a constant conductance per unit area. These results provide strong evidence that conductance changes are not necessary to activate exocytosis in these cells.

Methods. The increase of the membrane area during degranulation was monitored by measuring the membrane capacitance using the synthetic pseudo-random binary sequence (PRBS) method⁶. An 18-mV PRBS (128 bits long, 69 Hz bandwidth) was applied to the stimulus input of an EPC 7 patch-clamp amplifier (List electronics) operating in the voltage-clamp mode at a holding potential of 0 mV. The whole-cell current in response to the PRBS stimulus was sampled and stored by a PDP-11/23 minicomputer (Digital Equipment Corporation). The real part of the admittance was used to determine the capacitance C_m , the conductance $1/R_m$ of the plasma membrane, and the access resistance R_a . The d.c. conductance in *a* and *b* was determined by a fit of a quadratic function to the first eight data points at the low-frequency end of the admittance. After subtraction of this value, the frequency dependence can be linearized and C_m , G_m and R_a were then obtained by a linear least-squares fit. This procedure was carried out for each record (a record was taken every 1.8 s), giving the data points shown. The parameters obtained gave a good fit to the frequency dependence of the real and imaginary part of the admittance. All experiments were done at room temperature.



and conductance were determined every 1.8 s using a very low-frequency pseudo-random binary sequence technique (refs 1, 6; see Fig. 3 legend for details). After addition of DNP₄₃-BSA, the capacitance increased reproducibly by a factor of about 3 after a delay of 80 ± 20 s (Fig. 3*a*). The half-time for the rising phase was 20–60 s. In most, but not all, cases, the capacitance increase was preceded by a transient conductance increase in the nanosiemens range (Fig. 3*a*, lower trace). At 0 mV holding potential, this conductance change was associated with only a slight shift of the current in the outward direction. We suspected that this conductance increase resulted from the elevation of the intracellular free calcium concentration, which could lead to the opening of Ca-activated channels that have been observed in the mast cell membrane⁴. This transient conductance increase was not always seen, but on the other hand, Ca-activated channels were not observed in all cells⁴. In the presence of either quinidine (500 μ M) or pimozone (10 μ M), which have been reported to block Ca-activated channels in other cell types^{7,8}, the extent and time course of degranulation were unchanged, as shown by the measurement of the cell capacitance (Fig. 3*b*, upper trace), but no significant conductance changes were observed before the onset of degranulation (Fig. 3*b*, lower trace). In these experiments the membrane conductance averaged 95 ± 75 pS ($n = 4$) before degranulation started. The large scatter is probably due to different seal conductances. A small increase in the membrane conductance (from ~80 pS in the resting cell to 200 pS after degranulation; Fig. 3*b*) typically observed in

parallel with the increase in capacitance, probably reflects the increase in membrane area. This suggests that the specific conductance of the granule membrane is not very different from that of the plasma membrane.

It is generally believed that the opening of Ca-selective ion channels is an essential step in stimulus-secretion coupling. For example, conductance changes in the range 800–2,000 pS are required for exocytosis in adrenal chromaffin cells², the increase in conductance resulting from the opening of Ca-selective ion channels. Accordingly, Ca channels might open in response to the crosslinking of the IgE receptors in mast cells^{9,10}. In contrast to these expectations, our results show that rat peritoneal mast cells degranulate in response to antigenic stimulation without significant changes in membrane conductance.

Evidence for the entry of Ca as the main step in mast cell activation has been obtained from experiments showing that labelled Ca is taken up by degranulating mast cells¹¹, and that removal of extracellular Ca blocks antigen-induced degranulation¹². However, the large increase in membrane area that occurs during degranulation can provide new binding sites for the labelled Ca, which may account for the Ca uptake observed¹³. In our hands, the effect of removing Ca from the extracellular medium is time-dependent. If the cells are stimulated within 1 min of changing to a Ca-free extracellular solution, degranulation still occurs. Independence from extracellular Ca was also observed in experiments using intracellular Ca indicators which showed that mast cells stimulated 1 min after removal of

extracellular Ca produced a significant intracellular Ca transient in response to antigens¹⁴. The long-term effects of the removal of extracellular Ca probably result from the unbinding of Ca from plasma membrane sites, which could damage the membrane components involved in stimulus-secretion coupling.

The results presented here, and the lack of effect of calcium antagonists¹⁵ and of the removal of extracellular Ca at short times (ref. 14, also see above) argue against Ca entry as an obligatory event leading to exocytosis in mast cells.

We thank Dr H. Metzger for the gift of monoclonal IgE and its antigen, also Drs E. Neher, B. Sackmann, R. Eckert, M. Primke, A. Fahr and Mr J. Schroeder for discussions and comments on the manuscript. J.M.F. is an Alexander von Humboldt fellow.

Received 30 July; accepted 1 November 1985.

1. Fernandez, J. M., Neher, E. & Gomperts, B. D. *Nature* **312**, 453-455 (1984).
2. Neher, E. & Marty, A. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6712-6716 (1982).
3. Bennet, J. P., Cockcroft, S. & Gomperts, B. D. *J. Physiol., Lond.* **317**, 335-345 (1981).
4. Lindau, M. & Fernandez, J. M. *J. gen. Physiol.* (submitted).
5. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85-100 (1981).
6. Fernandez, J. M., Bezanilla, F. & Taylor, R. E. *J. gen. Physiol.* **79**, 41-67 (1982).
7. Fishman, M. C. & Spector, I. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5245-5249 (1981).
8. Hoffman, E. K., Simonsen, L. O. & Lambert, I. H. *J. Membrane Biol.* **78**, 211-222 (1984).
9. Gomperts, B. D. *Nature* **306**, 64-66 (1983).
10. Mazurek, N., Schindler, H., Schuerholz, T. H. & Pecht, I. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6841-6845 (1984).
11. Foreman, J. C., Hallet, M. B. & Mongar, J. I. *J. Physiol., Lond.* **271**, 193-214 (1977).
12. Foreman, J. C. & Mongar, J. I. *J. Physiol., Lond.* **224**, 753-769 (1972).
13. Grosman, N. & Diamant, B. *Trends Pharmac. Sci.* **5**, 183-184 (1984).
14. White, J. R., Ishizaka, T., Ishizaka, K. & Sha'afi, R. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3978-3982 (1984).
15. Ennis, M., Ind, P. W., Pearce, F. L. & Dollery, C. T. *Ag. Actions* **13**, 144-148 (1983).

Murine cells expressing an HLA molecule are specifically lysed by HLA-restricted antiviral human T cells

E. Gomard*, B. Begue*, S. Sodoyer†, J. L. Maryanski‡, B. R. Jordan† & J. P. Levy*

* INSERM, U 152, Bâtiment Gustave Roussy, Hôpital Cochin, 27 rue du Faubourg Saint-Jacques, 75014 Paris, France
 † Centre d'Immunologie, INSERM-CNRS de Marseille-Luminy, Case 906, Marseille Cedex 9, France
 ‡ Ludwig Institute for Cancer Research, Brussels Branch, 74 Avenue Hippocrate, UCL 7459, B-1200, Brussels, Belgium

Class I HLA (histocompatibility locus antigen) molecules are the targets of allospecific cytolytic T lymphocytes (CTL) in graft rejection, and constitute the restricting elements necessary for the interaction between antiviral CTL and virus-infected cells. Cells expressing only one HLA in the absence of other human molecules would provide a remarkable model for studying the function of these molecules. However, HLA⁺ murine cells transfected with human genes¹⁻⁵ are generally not lysed by allospecific human CTL⁵⁻⁸, and this is ascribed to insufficient HLA expression, lack of human β_2 -microglobulin, alteration of HLA molecules or absence of receptors for human T8 or LFA1 molecules in murine cells⁶⁻⁸. Here we report, for the first time, the specific lysis of virus-infected HLA⁺ murine cells by HLA-restricted antiviral human CTL. Therefore, these murine cells constitute an excellent model for studying the role of HLA molecules.

Murine cells expressing HLA-A2 or HLA-A11 molecules have been obtained by transfection of P815-HTR cells⁹ with genomic clones of well-established HLA specificities described previously^{10,11}. These cells were infected with the A/Texas (AT) human influenza A virus and successful infection was ascertained in a control experiment in which the cells were specifically

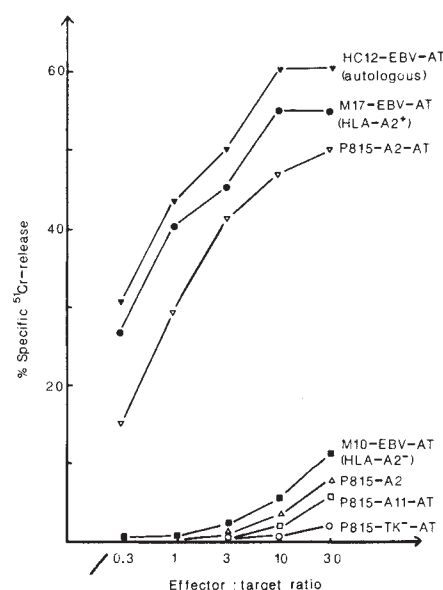


Fig. 1 Cytolytic activity of HLA-A2-restricted anti-influenza A human CTL on human or murine target cells. P815-HTR cells were transfected with either an HLA-A2 genomic clone¹⁰ by the calcium phosphate DNA precipitation method¹⁸, or an HLA-A11 clone¹¹ by the method of protoplast fusion in suspension¹⁹. HLA expression was ascertained by immunofluorescence using a monomorphic HLA-specific monoclonal antibody (B9.12.1). Lymphoblastoid cell lines were used as human target cells, as they are suitable targets and are easily infected with influenza virus¹². The target cells were autologous (HC12), HLA-A2-compatible (M17) or HLA-incompatible (M10) with the effector cells. Effector cells were obtained from an HLA-A2 donor (HC12) whose peripheral blood lymphocytes had been stimulated three times *in vitro* with influenza AT-infected autologous lymphocytes in the presence of interleukin-2, as described previously¹². The background lysis of target cells alone was between 20 and 24% for P815 cells whether infected or not, and between 15 and 26% for the human LCL target cells. The ⁵¹Cr-release test was performed after incubation at 37 °C for 4 h.

lysed by syngeneic anti-influenza murine CTL (data not shown). Uninfected HLA-A2⁺ and HLA-A11⁺ P815-HTR cells or untransfected P815-HTR cells were used as controls. Human anti-influenza A CTL of high efficiency were obtained from blood lymphocytes of donors after several *in vitro* stimulations with the virus, as described previously¹². CTL from donor HC12 lysed the autologous infected cells (HC12-EBV-AT) and similar degrees of lysis were found with other infected HLA-A2 target cells, as illustrated here by one example (M17-EBV-AT). HLA-incompatible infected target cells (M10-EBV-AT) were not lysed, as determined in several control experiments. As shown in Fig. 1 (and confirmed in five different experiments), the same CTL lysed with high efficiency the murine HLA-A2⁺ cells (P815-A2-AT) infected with influenza A virus. In fact, a similar level of lysis was observed with both human and murine HLA-A2⁺ target cells. Uninfected HLA-A2⁺ control P815 cells (P815-A2) were not lysed, confirming the viral specificity of the cytotoxicity. Furthermore, P815-A2 cells infected with influenza B Singapore virus were not lysed (data not shown).

To examine whether the lysis was HLA-restricted, untransfected infected P815-HTR cells (P815-AT) and HLA-A11⁺ infected P815-HTR cells (P815-A11-AT) were tested with human HLA-A2-restricted virus-specific CTL. These cells showed no lysis (Fig. 1). However, aliquots of P815-AT and P815-A11-AT cells were lysed very specifically by either murine anti-influenza CTL or murine anti-H-2^d CTL (not shown), confirming that these targets were virus-infected and lysable. Unfortunately, it was not possible to test HLA-A11-restricted human CTL, as HLA-A11 is a poor restricting element of anti-influenza responses in

Table 1 Treatment of effector cells with anti-T3, anti-T4 or anti-T8 monoclonal antibody (MAb) plus complement (C)

Treatment of effector cells	% Specific ⁵¹ Cr-release by:	
	M17-EBV-AT	P815-A2-AT
—	37	41
Anti-T3 MAb+C	<2	2
Anti-T4 MAb+C	36	40
Anti-T8 MAb+C	<2	<2

HC12 anti-influenza AT effector cells were incubated for 30 min at 4°C with OKT3, OKT4 or OKT8 monoclonal antibody (Ortho) diluted 1:100, then rabbit complement (Behring Institut) diluted 1:20 was added and the mixture incubated for 45 min at 37°C, after which the cells were washed and resuspended in test medium. The effector/target cell ratio (calculated for effector cells before treatment) was 0.5:1.

man¹³. However, we have demonstrated that an influenza A-specific human CTL clone from the same donor but restricted by HLA-DRw8 (ref. 14) did not lyse P815-A2-AT cells. Furthermore, uncloned anti-influenza CTL restricted by HLA-B27 did not lyse P815-A2-AT (not shown). These results show that the human CTL used here lysed the P815 target cells not only in a virus-specific but also in an HLA-restricted manner, and similar results have been found with different batches of human effector cells, including CTL obtained after secondary stimulation *in vitro* and before their establishment as a permanent cell line.

The T4⁺T8⁺ phenotype of the human anti-influenza A CTL has been well established. To further confirm this identity for the present study, the effector cells were treated with anti-T3, anti-T4 or anti-T8 monoclonal antibody plus complement just before the chromium release test. Treatment with anti-T3 or anti-T8 antibody plus complement either abolished or strongly decreased the cytolysis of P815-A2-AT by human CTL, while anti-T4 had no effect (Table 1).

The present results show that murine cells infected with influenza A virus and expressing the HLA-A2 heavy chain can be lysed by HLA-A2-restricted anti-influenza A CTL of human origin as efficiently as the relevant human targets. The interaction is clearly virus-specific and HLA-A2-restricted, and the effector cells belong to the T8 subset of T cells. These results are in contrast to the negative or marginally positive results of experiments reported recently by several groups, including ourselves, in which anti-HLA human CTL were tested on HLA-transfected murine L-cells⁶⁻⁸. The discrepancies between these results may be caused by a number of factors. First, P815 cells are intrinsically better targets than L-cells when tested with murine CTL and, more significantly, with human allospecific CTL directed against an HLA molecule expressed on the (transfected) P815 target; this has been observed with P815-HLA-A24 cells¹⁵ and we have found that P815-A2 and P815-A11 cells are lysed quite specifically by human anti-A2 or anti-A11 CTL, respectively¹⁶. However, a good degree of lysis has been observed recently with L-cells expressing a high concentration of a variant HLA-A3 antigen¹⁷, which suggests that quantitative rather than qualitative factors may limit the interaction of human CTL with murine target cells. Another point to consider is that antiviral responses have been studied in the present experiments. In such reactions, the HLA epitope involved may be different from the epitopes recognized in anti-HLA responses. Moreover, simultaneous recognition of both HLA (restricting element) and viral protein by the T-cell receptor(s) may result in higher affinity. On the whole, and in contrast to previous suggestions⁶⁻⁸, it is clear that murine cells transfected with human HLA genes provide a suitable model for the study of the relationship between the structure and function of human HLA molecules.

We thank Drs H. Orr and P. Paul for providing HLA-A2 and HLA-A11 genomic clones, Dr A. Van Pel for the gift of P815-HTR cells, and Mrs J. Debie for secretarial help.

Received 16 July; accepted 23 October 1985.

1. Lemonnier, F. A. *et al. Immunogenetics* **16**, 355-362 (1982).
2. Barbosa, J. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 6327-6331 (1982).
3. Kavathas, P. & Herzenberg, L. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 524-528 (1983).
4. Herman, A., Parham, P., Weissman, S. M. & Engelhard, V. H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5056-5060 (1983).
5. Barbabeu, C. *et al. J. Immun.* **131**, 2032-2037 (1983).
6. Barbosa, J. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 7549-7553 (1984).
7. Van de Rijn, M. *et al. Science* **226**, 1083-1085 (1984).
8. Barbabeu, C. *et al. J. Immun.* **133**, 3188-3196 (1984).
9. Van Pel, A., De Plaen, E. & Boon, T. *Somat. Cell molec. Genet.* (in the press).
10. Koller, B. H. & Orr, H. T. *J. Immun.* **134**, 2727-2733 (1985).
11. Paul, P. *et al. Immunogenetics* (in the press).
12. Toubert, A. *et al. Immunogenetics* **20**, 513-525 (1984).
13. Gomard, E., Witbon, M., Toubert, A., Begue, B. & Lévy, J. F. *Immunogenetics* **20**, 197-204 (1984).
14. Henin, Y. *et al. Immunogenetics* (in the press).
15. Maryanski, J. L. *et al. Eur. J. Immun.* (in the press).
16. Achour, A. *et al. Eur. J. Immun.* (submitted).
17. Cowan, E. P., Coligan, J. E. & Biddison, W. E. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
18. Wigier, M., Pellicer, A., Silverstein, S. & Axel, R. *Cell* **14**, 725-731 (1978).
19. Litzkas, P., Jha, K. K. & Oze, H. L. *Molec. cell. Biol.* **4**, 2549-2552 (1984).

A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes

Harinder Singh*, Ranjan Sen†, David Baltimore*† & Phillip A. Sharp*

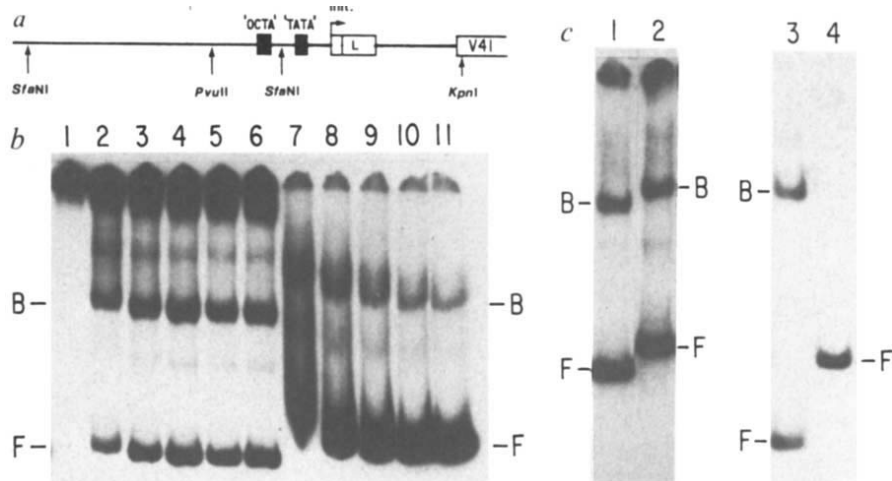
* Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

† Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA

Trans-acting factors that mediate B-cell specific transcription of immunoglobulin genes have been postulated based on an analysis of the expression of exogenously introduced immunoglobulin gene recombinants in lymphoid and non-lymphoid cells. Two B-cell-specific, cis-acting transcriptional regulatory elements have been identified. One element is located in the intron between the variable (V) and constant (C) regions of both heavy and κ light-chain genes and acts as a transcriptional enhancer¹⁻⁶. The second element is found upstream of both heavy and κ light-chain gene promoters. This element directs lymphoid-specific transcription even in the presence of viral enhancers⁷⁻¹⁰. We have sought nuclear factors that might bind specifically to these two regulatory elements by application of a modified gel electrophoresis DNA binding assay¹¹⁻¹³. We report here the identification of a human B-cell nuclear factor (IgNF-A) that binds to DNA sequences in the upstream regions of both the mouse heavy and κ light-chain gene promoters and also to the mouse heavy-chain gene enhancer. This sequence-specific binding is probably mediated by a highly conserved sequence motif, ATTTGCAT, present in all three transcriptional elements. Interestingly, a factor showing similar binding specificity to IgNF-A is also present in human HeLa cells.

Mouse and human light-chain promoters contain the octamer sequence ATTTGCAT approximately 70 base pairs (bp) upstream from the site of initiation^{14,15}. Heavy-chain gene promoters contain the identical sequence in inverted orientation, ATGCAAAT, at the same position. This element seems to be essential for the efficient use of immunoglobulin promoters in B cells^{5,7,15}. The high degree of sequence and positional conservation of this element as well as its apparent functional requirement suggests its interaction with a sequence-specific transcription factor. We have searched for such a factor in a B-cell nuclear extract by using a gel electrophoresis DNA binding assay with enhanced sensitivity. This assay, which is based on the altered mobility of protein-DNA complexes during gel electrophoresis, has been extensively used in equilibrium and kinetic analyses of purified prokaryotic gene regulatory proteins^{11,12,16,17} and more recently to identify and isolate a protein

Fig. 1 Binding of a nuclear factor to the MOPC-41 κ promoter region. **a**, 5' region of the MOPC-41 κ gene segment. The 'OCTA' and 'TATA' elements start at positions -66 and -28, respectively. **b**, Gel electrophoresis DNA binding assays with the *Sfa*NI/*Sfa*NI κ promoter fragment (~300 bp). The *Sfa*NI fragment was subcloned into the *Sma*I site of pSP64 (pSP1gV κ , provided by N. Speck). For binding analysis, this fragment was excised from pSP1gV κ by digesting with *Hind*III and *Eco*RI. These latter sites flank the *Sma*I site in the polylinker of pSP64. After end-labelling with [α -³²P]dATP and the large fragment of *E. coli* DNA polymerase I, the radiolabelled fragment was isolated by polyacrylamide gel electrophoresis. The ³²P-labelled fragment (~0.5 ng, 10,000 c.p.m.) was incubated with a nuclear extract of a human B lymphoma cell line (EW³²) in the absence (lane 1) or presence of two different non-specific competitor DNAs (lanes 2-11). Binding reactions (25 μ l) contained 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol and 8 μ g EW nuclear extract protein. Reactions 2-6 also contained 800, 1,600, 2,400, 3,200 and 4,000 ng, respectively, of poly(dI-dC)·poly(dI-dC). Reactions 7-11 contained 300, 600, 900, 1,200 and 1,500 ng, respectively, of *Hinf*I-digested *E. coli* chromosomal DNA. After 30 min incubation at room temperature, the resulting complexes were resolved in a low-ionic-strength 4% polyacrylamide gel (acrylamide:bisacrylamide weight ratio of 30:1) containing 6.7 mM Tris-HCl (pH 7.5), 3.3 mM Na-acetate and 1 mM Na-EDTA¹³. The gel was pre-electrophoresed for 30 min at ~11 V cm⁻¹. Electrophoresis was carried out at the same voltage gradient for 90 min at room temperature with buffer recirculation. The gel was then dried and autoradiographed at -70 °C with a screen. F and B indicate positions of free and bound fragments, respectively. **c**, Binding assays as detailed in **b** using 2,400 ng poly(dI-dC)·poly(dI-dC) and the following DNA fragments: κ *Sfa*NI/*Sfa*NI (~0.5 ng, 10,000 c.p.m., lane 1), κ *Pvu*II/*Kpn*I (~0.5 ng, 10,000 c.p.m., lane 2), κ *Pvu*II/*Sfa*NI (~0.1 ng, 5,000 c.p.m., lane 3) and pSP64 *Pvu*II/*Eco*RI (~0.2 ng, 5,000 c.p.m., lane 4). The κ *Pvu*II/*Sfa*NI fragment was derived from the plasmid pSP1gV κ by digesting with *Pvu*II and *Eco*RI. The *Eco*RI site is in the polylinker and therefore this fragment contains 16 bp of polylinker sequence.



that binds to satellite DNA from a nuclear extract of monkey cells¹³. In the latter study an excess of heterologous competitor DNA (*Escherichia coli*) was included with the specific probe fragment to bind the more abundant nonspecific DNA binding proteins in the extract. To minimize the binding of sequence-specific proteins to such heterologous sequence competitor DNA, we tested as a replacement the simple alternating co-polymer duplex poly(dI-dC)·poly(dI-dC).

A radiolabelled *Sfa*NI/*Sfa*NI DNA fragment derived from the upstream region of the MOPC 41 κ light-chain gene (Fig. 1a) was incubated with a nuclear extract of a human B-cell line in the absence or presence of two competitor DNAs. The resulting complexes were resolved from the free fragment by electrophoresis through a low-ionic strength, non-denaturing polyacrylamide gel and visualized by autoradiography (Fig. 1b). In the absence of competitor DNA, all of the labelled fragment was retained at the top of the gel (Fig. 1b, lane 1), probably due to the binding of an excess of non-sequence-specific proteins. With addition of increasing amounts of either poly(dI-dC)·poly(dI-dC) (Fig. 1b, lanes 2-6) or *E. coli* chromosomal DNA (lanes 7-11) as competitors, putative protein-DNA complexes which migrated more slowly than the free fragment were detected. The relative abundance of the major species of complex (B) as well as that of minor species was significantly greater in the presence of the alternating co-polymer competitor DNA. Because substitution of the simple co-polymer considerably increased the sensitivity of the assay, it has been used in all subsequent binding analyses.

To test the sequence specificity of the major species detected in the binding assay, a set of mutually overlapping κ promoter fragments (Fig. 1a, *Sfa*NI/*Sfa*NI, *Pvu*II/*Kpn*I and *Pvu*II/*Sfa*NI) and a similar-length fragment derived from the bacterial plasmid SP64 (*Eco*RI/*Pvu*II) were individually assayed (Fig. 1c). Whereas the bacterial DNA fragment showed no appreciable binding (Fig. 1c, lane 4), all of the κ promoter fragments yielded major discrete complexes of similar mobilities (lanes 1-3). We have found that the mobilities of a series of complexes formed with different-length fragment probes (75-300 bp) are approximately the same (data not shown). These data therefore suggested the binding of a specific nuclear factor within the region of overlap of the κ promoter fragments. This region includes the conserved octameric sequence but not the TATA element. Note that with the smallest 75-bp κ promoter

fragment (Fig. 1c, lane 3), no appreciable label was retained at the top of the gel. Thus, as has been noted recently, the use of small probe fragments further enhances the sensitivity of detection of specific protein-DNA complexes¹⁸.

The sensitivity gained by using both the co-polymer and a small fragment probe permitted the detection of two complexes, B1 and B2 (Fig. 2a, lane 1). The major species, B1, corresponds to complex B in Fig. 1. The relative affinity of the factor(s) for κ promoter DNA was estimated by a competition assay (Fig. 2a). Whereas a control plasmid (pSP64), when added in the above incubation, failed to compete for binding in the concentration range tested (Fig. 2a, lanes 2-4), the recombinant plasmid (pSP1gV κ) effectively reduced the formation of both B1 and B2 in the same range (lanes 5-7). The latter plasmid was constructed by insertion of the upstream region of the κ promoter into pSP64. Assuming that the pSP1gV κ plasmid contains a single high-affinity binding site, these results suggest that the nuclear factor(s) responsible for B1 and B2 has at least a 10⁴-fold higher affinity for its cognate sequence than for heterologous plasmid DNA (see Fig. 2a legend).

To determine if the factor(s) responsible for the formation of B1 and B2 is specific to B lymphocytes, a nuclear extract derived from human HeLa cells was assayed for binding to the κ promoter probe (Fig. 2b). Both B1 and B2 were generated at similar levels by the HeLa extract to those observed with B-cell extracts (Fig. 2b, lane 1); furthermore, both were specifically competed for by the pSP1gV κ plasmid (lane 2). Thus, HeLa cells also contain a factor(s) which binds specifically to the κ promoter upstream region.

DNase I footprint analysis was used to delineate at a higher resolution the binding domain(s) of factor(s) present in complexes B1 and B2^{19,20}. To facilitate these studies, the binding factor(s) from B cells was partially purified by chromatography of nuclear extract protein on a heparin-Sepharose column. Most of the binding activity eluted in a 0.25 M KCl step fraction, giving an approximately fivefold purification (data not shown; see Fig. 3 legend). For footprint analysis, after incubation of the partially purified factor(s) with the κ promoter probe, sufficient DNase I was added to produce a partial digestion of the DNA. The B1 and B2 species were then resolved from free fragment by polyacrylamide gel electrophoresis. Bound DNA was eluted from both B1 and B2 bands and examined by denaturing polyacrylamide gel electrophoresis (Fig. 3, lanes 2, 3 and 2',

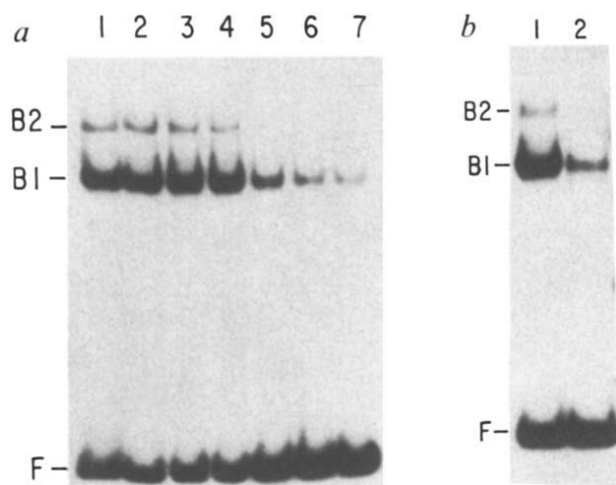


Fig. 2 Binding competition analysis in nuclear extracts of human EW and HeLa cells. **a**, EW nuclear extract. Binding assays as detailed in Fig. 1b legend using radiolabelled κ PvuII/SfaNI fragment (~ 0.1 ng, 5,000 c.p.m.) and 2,400 ng poly(dI-dC)·poly(dI-dC). Reactions 2–4 additionally contained 50, 100 and 200 ng, respectively, of the bacterial plasmid pSP64 whereas reactions 5–7 contained 50, 100 and 200 ng, respectively, of the recombinant plasmid pSPiV κ . Assuming that a molecule of pSPiV κ contains a single high-affinity site whereas a molecule of pSP64 (3,000 bp) contains 3,000 nonspecific sites, the apparent affinity ratio of the factor for these two types of sites is greater than $3,000 \times 200/50 = 1.2 \times 10^4$. **b**, HeLa nuclear extract. Binding assays as detailed in Fig. 1b legend using radiolabelled κ PvuII/SfaNI fragment (~ 0.1 ng, 5,000 c.p.m.), 2,400 ng poly(dI-dC)·poly(dI-dC) and 6 μ g HeLa nuclear extract protein³⁰ (provided by P. Grabowski). Reactions 1 and 2 additionally contained 100 ng of pSP64 and pSPiV κ , respectively.

3'). DNase I digests of the κ promoter probe in the absence of B-cell protein (Fig. 3, lanes 1 and 1') and A+G chemical cleavage ladders were co-electrophoresed to map the binding domain. Factor(s) in the B1 complexes (Fig. 3, lanes 2 and 2') appeared to protect a 19-nucleotide region on the coding strand. The 5' and 3' boundaries of the protected region map to positions –72 and –52, respectively, from the site of transcriptional initiation³. The region of DNase I protection was centred about the conserved octanucleotide sequence ATTTGCAT, suggesting its importance in the recognition of the immunoglobulin promoter by the nuclear factor. B2 complexes showed a virtually identical DNase I protection pattern to B1 complexes and therefore do not appear to involve additional DNA contacts (Fig. 3, lanes 3 and 3'). The simplest interpretation of this observation is that the B2 complex is generated by dimerization through protein-protein interactions of the factor responsible for the B1 complex. Alternatively, the B2 complex could be formed either by the binding of another protein to the factor responsible for the B1 complex or by recognition of the same set of sequences by a distinct DNA binding protein.

As the octamer sequence motif is present in both light- and heavy-chain gene promoters as well as in the enhancer elements of both mouse and human heavy-chain genes^{15,21}, we assayed for a factor binding to fragments from a mouse heavy-chain promoter (V_H) and the mouse heavy-chain enhancer. The V_H promoter fragment was derived from the 5' region of the V17.2.25 gene and included nucleotides between positions –154 and +57 relative to the transcriptional start site⁸. In this promoter the conserved octanucleotide spans positions –57 to –50 (Fig. 4a). The heavy-chain enhancer fragment was derived from the germline joining-constant (J_H-C_μ) region and spanned positions 81–251 within a 313-bp region implicated in enhancer function¹. The conserved octanucleotide is positioned between coordinates 166 to 173 in the above fragment (Fig. 4a). The B-cell heparin-Sepharose fraction (purified on the basis of binding to the κ promoter sequence; Fig. 4b, lane 1) produced a complex with both the V_H promoter fragment (lane 4) and the

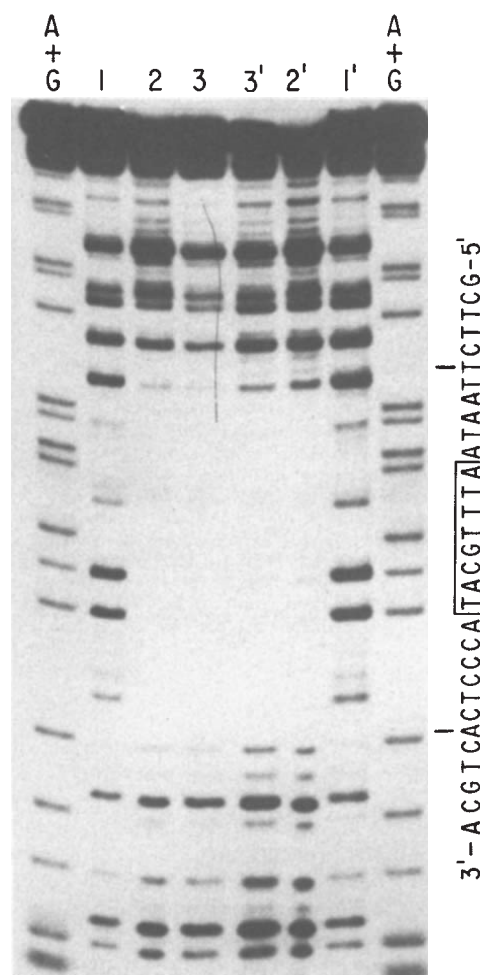


Fig. 3 DNase I footprinting of factor-DNA complexes. The B-cell nuclear extract was applied to a heparin-Sepharose column equilibrated with 20 mM HEPES pH 7.9, 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 5 mM MgCl₂ and 0.1 M KCl. The κ -promoter binding factor was eluted with a 0.25 M KCl step. Binding reactions with this fraction (30 μ l) contained 2.5 mM MgCl₂, κ PvuII/SfaNI (~ 1 ng, 100,000 c.p.m.) and 4,800 ng poly(dI-dC)·poly(dI-dC) in addition to components detailed in Fig. 1b legend. The coding strand of the κ promoter probe was 3' end-labelled (EcoRI site) with [α -³²P]dATP using the large fragment of *E. coli* DNA polymerase. Reaction 1 was digested with DNase I (5 μ g ml⁻¹) for 2.5 min at room temperature in the absence of B-cell nuclear protein. Reaction 2 was initially incubated with the heparin-Sepharose fraction of the EW nuclear factor (14 μ g protein) for 15 min at room temperature and then digested with DNase I as above. Each reaction was stopped with EDTA (5 mM) and the products separated by native polyacrylamide gel electrophoresis as detailed for Fig. 1b. After autoradiography to visualize the various species, DNA was eluted from the free (reaction 1) and bound (B1 and B2, reaction 2) fragment bands by incubating gel slices in 0.5 M ammonium acetate (pH 7.5), 0.1% SDS and 1 mM EDTA with shaking at 37 °C overnight. The supernatants were extracted sequentially with phenol/chloroform/isoamylalcohol (25:24:1 v/v) and chloroform/isoamylalcohol (24:1 v/v) and precipitated with 2 vol ethanol in the presence of carrier transfer RNA. After a re-precipitation step, the products were analysed by separation in a 10% polyacrylamide gel (20:1) in the presence of 8 M urea followed by autoradiography at –70 °C with a screen. Lane 1 contains products of free fragment digestion from reaction 1. Lanes 2 and 3 contain digestion products eluted from bound fragment bands B1 and B2, respectively, from reaction 2. Lanes 1', 2' and 3' correspond to 1, 2 and 3, respectively, except that the former set was digested with DNase I for 5 min. A+G chemical cleavage ladders³¹ of the κ promoter probe were co-electrophoresed to map the binding domain.

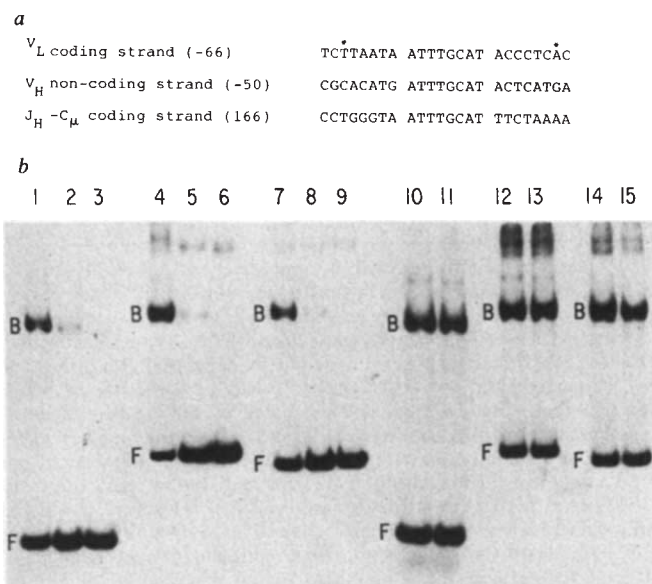


Fig. 4 Binding of a common nuclear factor to three immunoglobulin transcriptional control elements. **a**, Nucleotide sequences of actual and putative binding sites. The V_L binding site is defined by the DNase I protection assay (* indicates boundaries of the protected region). The V_H and J_H - C_μ sequence are putative binding sites in the $V_{H2.25}$ promoter⁸ and the mouse heavy-chain enhancer¹, respectively. Numbers in parentheses indicate start coordinates of octamer motif. **b**, Binding competitions. Binding assays (10 μ l) as detailed in Fig. 1b legend using 1,600 ng poly(dI-dC)·poly(dI-dC) and the heparin-Sepharose fraction of the EW nuclear factor (1.5 μ g protein). V_L probe (~0.1 ng, 5,000 c.p.m.), lanes 1-3, 10, 11. V_H probe (~0.2 ng, 5,000 c.p.m.), lanes 4-6, 12, 13. J_H - C_μ probe (~0.2 ng, 5,000 c.p.m.), lanes 7-9, 14, 15. Lanes 2, 5, 8 additionally contained 5 ng of a V_L promoter oligomer (36 bp, spanning positions -81 to -44 of the MOPC-41 V_κ gene segment³) whereas lanes 3, 6, 9 contained 50 ng of the same oligomer. Lanes 11, 13, 15 additionally contained 50 ng of a J_H - C_μ oligomer that lacks the octamer motif (41 bp, spanning positions -1 to 40 of the heavy-chain enhancer^{1,2}). Complementary single-stranded synthetic oligonucleotides were kindly made by Dr Ronald Mertz, Genzentrum der Universität München and Dr E.-L. Winnacker, Institut für Biochemie der Universität München. They were annealed before use as competition substrates in the binding assay.

enhancer fragment (lane 7). The mobilities of the complexes formed with the three fragments were very similar, consistent with the binding of a common factor. Furthermore, binding to these fragments was specifically competed for by a synthetic duplex 40-mer (Fig. 4b legend) that spanned the octanucleotide motif of the MOPC 41 κ light-chain gene promoter (lanes 1-9). An oligomer of equivalent size containing a sequence from a region of the mouse heavy-chain enhancer lacking the octanucleotide motif (Fig. 4b legend) failed to compete for binding in the same concentration range (lanes 10-15). This competition analysis further strengthens the suggestion that a common nuclear factor (IgNF-A) binds to all three immunoglobulin transcriptional elements. As mentioned above these three transcriptional elements share an identical sequence motif, ATTTGCAT (Fig. 4a). Thus, the binding of a common nuclear factor is almost certainly mediated by this motif.

The use of a sensitive gel electrophoresis DNA binding assay in conjunction with the co-polymer competitor poly(dI-dC)·poly(dI-dC) has facilitated the identification of a putative mammalian gene regulatory factor. The simple alternating co-polymer probably competes less effectively than heterologous sequence DNA for binding of a sequence-specific factor, thereby significantly increasing the sensitivity of the assay. The synthetic co-polymer has proved similarly useful in the identification of other immunoglobulin enhancer binding factors (R.S. *et al.*, in

preparation) and also a transcription factor that binds to an upstream sequence in the adenovirus major late promoter²². A further increase in sensitivity in this assay is obtained by the use of small DNA probes (<100 bp) which minimize nonspecific binding interactions in a crude extract¹⁸. Binding competitions and DNase I footprinting analysis can be used to test the affinity and sequence specificity of the protein-DNA complexes. In the future, relevant factors can be purified using this assay for structure-function analyses. Such an approach will greatly facilitate the elucidation of the set of DNA binding mammalian gene regulatory proteins.

The biological function(s) of IgNF-A and its biochemical mode(s) of action remain unknown. Since deletion or disruption of the IgNF-A binding site in immunoglobulin promoters significantly reduces the level of accurately initiated transcripts *in vivo*^{5,7,15} and *in vitro* (R.S. *et al.*, in preparation), we propose that IgNF-A is a sequence-specific positive *trans*-acting factor. Its mode of action may be similar to the eukaryotic promoter-specific transcription factors Spl (refs 23) and heat-shock transcription factor (HSTF)²⁴.

The IgNF-A binding site seems to be a functional component of the B lymphocyte-specific immunoglobulin promoter⁷. For example, sequences from this promoter containing the IgNF-A binding site specify accurate transcription in B cells but not in HeLa cells. The role of IgNF-A in this B-cell specificity is unclear, since point mutations in the IgNF-A binding site have not been tested *in vivo* to determine whether this sequence is responsible for such specificity. IgNF-A may not be restricted to B cells, as a factor has been detected in HeLa cell extracts which generates complexes with similar mobilities and sequence specificity (as tested by competition analysis). However, further experiments will be necessary to show whether the factor in HeLa extracts is similar or identical to IgNF-A. Interestingly, the immunoglobulin octamer motif in the IgNF-A binding site has recently been shown to be present in the upstream region (about -225 bp) of vertebrate U1 and U2 small nuclear RNA genes²⁵⁻²⁸. More importantly, this element dramatically stimulates (20-50-fold) transcription of U2 snRNA genes in *Xenopus* oocytes^{27,28}. Therefore, IgNF-A may be a constitutive activator protein that also functions in the high-level expression of U1 and U2 snRNA genes in vertebrate cells.

The presence of an IgNF-A binding site in the mouse heavy-chain enhancer is intriguing. It suggests the additional involvement of IgNF-A in enhancer function. Deletion of an 80-bp region of the enhancer containing the putative binding site reduces enhancer activity ~10-fold¹. The occupation of this binding site, *in vivo*, has been inferred from the fact that the G residue in the enhancer octamer is protected from dimethyl sulphate modification only in cells of the B lineage²⁹. Furthermore, IgNF-A also binds in a sequence-specific manner to the simian virus 40 enhancer (J. Weinberger, personal communication), which contains the immunoglobulin octamer motif¹⁵, thereby strengthening the notion that the factor participates in enhancer function.

This work was supported by grants from NIH (R01-GM32467, R01-CA38660), partially from an NCI Cancer Center Support (core) (P30-CA14051), from NSF (PCM-8200309) to P.A.S. and by a grant from the American Cancer Society (IM-355Q) to D.B. H.S. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research. R.S. is a fellow of the Damon Runyon-Walter Winchell Cancer Fund. We thank Drs J. Weinberger and J. Lebowitz for helpful discussions and a critical reading of the manuscript.

Received 16 September; accepted 5 November 1985.

1. Banerji, T., Olson, L. & Schaffner, W. *Cell* **33**, 729-740 (1983).
2. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. *Cell* **33**, 717-728 (1983).
3. Queen, C. & Baltimore, D. *Cell* **33**, 741-748 (1983).
4. Picard, D. & Schaffner, W. *Nature* **307**, 80-82 (1984).
5. Bergman, Y., Rice, D., Grosschedl, R. & Baltimore, D. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7041-7045 (1984).

6. Potter, H., Weir, L. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7161-7165 (1984).
7. Mason, J. O., Williams, G. T. & Neuberger, M. S. *Cell* **41**, 479-487 (1985).
8. Grosschedl, R. & Baltimore, D. *Cell* **41**, 885-897 (1985).
9. Falkner, F. G., Neumann, E. & Zachau, H. *Hoppe-Seyler's Z. physiol. Chem.* **365**, 1331-1334 (1984).
10. Foster, J., Stafford, J. & Queen, C. *Nature* **315**, 423-425 (1985).
11. Fried, M. & Crothers, D. M. *Nucleic Acids Res.* **9**, 6505-6525 (1981).
12. Garner, M. M. & Revzin, A. *Nucleic Acids Res.* **9**, 3047-3060 (1981).
13. Strauss, F. & Varshavsky, A. *Cell* **37**, 889-901 (1984).
14. Parslow, T. G., Blair, D. L., Murphy, W. J. & Granner, D. K. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2650-2654 (1984).
15. Falkner, F. G. & Zachau, H. G. *Nature* **310**, 71-74 (1984).
16. Fried, M. G. & Crothers, D. M. *J. molec. Biol.* **172**, 241-262, 263-282 (1984).
17. Hendrickson, W. & Schleif, R. F. *J. molec. Biol.* **174**, 611-628 (1984).
18. Schneider, R., Dörper, Th., Gander, I., Mertz, R. & Winnacker, B.-L. *EMBO J.* (in the press).
19. Galas, D. & Schmitz, A. *Nucleic Acids Res.* **5**, 3157-3170 (1978).
20. Topol, J., Ruden, D. N. & Parker, C. S. *Cell* **42**, 527-537 (1985).
21. Hayday, A. C. *et al. Nature* **307**, 334-340 (1984).
22. Carthew, R. W., Chodosh, L. A. & Sharp, P. A. *Cell* **43**, 439-448 (1985).
23. Dynan, W. S. & Tjian, R. *Cell* **32**, 669-680 (1983); **35**, 79-87 (1983).
24. Parker, C. S. & Topol, J. *Cell* **37**, 273-283 (1984).
25. Krol, A., Lund, E. & Dahlberg, J. E. *EMBO J.* **6**, 1529-1535 (1985).
26. Ciliberto, G., Buckland, R., Cortese, R. & Philipson, L. *EMBO J.* **6**, 1537-1543 (1985).
27. Ares, M. Jr, Mangin, M. & Weiner, A. M. *Molec. cell. Biol.* **5**, 1560-1570 (1985).
28. Mattaj, I. W., Leinherd, S., Jiricny, J. & DeRobertis, E. M. *Nature* **316**, 163-167 (1985).
29. Ephrussi, A., Church, G. M., Tonegawa, S. & Gilbert, W. *Science* **227**, 134-140 (1985).
30. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. *Nucleic Acids Res.* **11**, 1475-1489 (1983).
31. Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499-525 (1980).
32. Magrath, I. T. *et al. J. natn. Cancer Inst.* **64**, 465-476 (1980).

Activated human monocytes express the c-sis proto-oncogene and release a mediator showing PDGF-like activity

Yves Martinet, Peter B. Bitterman*,
Jean-Francois Mornex*, Gary R. Grotendorst*,
George R. Martin & Ronald G. Crystal

Pulmonary Branch, National Heart, Lung, and Blood Institute, and Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892, USA

Current ideas about the mechanism of wound healing and the pathogenesis of atherosclerosis, pulmonary fibrosis and hepatic fibrosis suggest a central role for the mononuclear phagocyte in attracting and/or stimulating the proliferation of mesenchymal cells¹⁻⁵. We demonstrate here that activated human blood monocytes, but not resting monocytes, release a mediator that attracts smooth muscle cells and cooperates with other mediators to stimulate fibroblast proliferation. This mediator is very similar to platelet-derived growth factor (PDGF): its chromatographic properties and chemical stability are similar to those of PDGF^{6,7}, it competes with ¹²⁵I-PDGF for binding to fibroblasts and it immunoprecipitates with anti-PDGF antibodies. In parallel, stimulated monocytes, but not resting monocytes, express the c-sis proto-oncogene, a gene coding for one of the PDGF chains^{8,9}, consistent with the concept that expression of the c-sis proto-oncogene may be involved in the ability of mononuclear phagocytes to modulate the accumulation of mesenchymal cells.

The idea that activated human blood monocytes release a mediator possessing PDGF-like activity evolved from the demonstration that blood monocytes can release a mediator that attracts smooth muscle cells (SMC) and cooperates with other mediators in stimulating fibroblasts to proliferate (Fig. 1). When activated, monocytes released into the media a mediator that attracted SMC in a concentration-gradient-dependent fashion. This was observed independently of the stimulus used; opsonized zymosan, immune complexes and lipopolysaccharide all activated monocytes to release this mediator in a time-dependent fashion, the activity increasing up to 4 h and then levelling off. In contrast, the release of SMC chemotactic activity by unstimu-

Table 1 Biochemical characteristics of the mediator released by activated monocytes

Conditions	% Activity
Heat (100 °C, 10 min)	80 ± 4
Acid (pH 2.5, 22 °C, 30 min)	63 ± 5
Reduction (2 mM 2-mercaptoethanol, 22 °C, 30 min)	0
Trypsin (5 × 10 ⁻⁶ M, 37 °C, 30 min)	24 ± 2

Monocytes were cultured with lipopolysaccharide B (LPS) for 24 h at 37 °C, and the supernatant was tested for SMC chemotactic activity as indicated in Fig. 1a. Before exposure to various treatments, the supernatant chemotactic activity was equal to 290 ± 37 chemotactic units. Aliquots (1 ml) were exposed to various experimental conditions and the chemotactic activity was quantified as the per cent activity before any treatment. Heat stability was evaluated by boiling the monocyte supernatant for 10 min, cooling to 23 °C and then testing for chemotactic activity. Acid stability was determined by dialysing 1 ml of the monocyte supernatant overnight against 0.5 M acetic acid, lyophilizing and redissolving it with HCl (to a final molarity of 10 mM) before diluting in culture media for testing for chemotactic activity. Sensitivity to reduction was evaluated by dialysing the monocyte supernatant overnight against 100 mM Tris-HCl pH 8.0; 2-mercaptoethanol was then added and the supernatant incubated for 30 min. The supernatant was then dialysed against culture medium overnight and tested for chemotactic activity. Sensitivity to trypsin was evaluated by adding trypsin (100 µg, Worthington) to 1 ml of monocyte supernatant. After 30 min at 37 °C, soybean trypsin inhibitor (200 µg, Worthington) was added and the supernatant tested for chemotactic activity. At the concentration used, the soybean trypsin inhibitor mixture with or without trypsin did not alter the activity of the original monocyte supernatant. All studies were performed in triplicate and the data are presented as mean ± s.d.

lated cells was minimal. The release of the SMC chemotactic signal by stimulated monocytes was completely inhibited by cycloheximide (not shown), suggesting it was synthesized by monocytes in response to the activation stimulus. Following partial purification by carboxymethyl-cellulose and blue-Sepharose chromatography, the monocyte mediator with SMC chemotactic activity was also demonstrated to have growth activity for human diploid lung fibroblasts and BALB/c-3T3 fibroblasts. Importantly, the growth factor activity had characteristics of a competence factor, that is, although it did not by itself stimulate the growth of fibroblasts, it did so in the presence of the progression factor insulin. Furthermore, in all samples, this paralleled the chemotactic activity for SMC.

Although several biological compounds are known to be chemotactic for mesenchymal cells¹⁰, only two, PDGF¹¹ and fibronectin¹², are known also to have growth-competence activity for fibroblasts. Mononuclear phagocytes produce fibronectin, but when the supernatant from activated monocytes was chromatographed on gelatin-Sepharose to remove fibronectin, none of the SMC chemotactic activity was removed. Furthermore, the chemotactic activity present in conditioned media was not inhibited by the addition of antibodies to fibronectin (not shown). Finally, the conclusion that this monocyte mediator possessing SMC chemotactic activity and growth-competence activity was not fibronectin is consistent with the finding that activated human blood monocytes release maximal amounts of the PDGF-like molecule within 4 h of culture but release significant amounts of fibronectin only after 48 h of culture¹³.

Further evidence that this monocyte mediator is PDGF-like came from: (1) its behaviour following exposure to various conditions (Table 1); it was resistant to heat and acid, but was sensitive to proteolysis and its activity was completely abolished by reduction, suggesting that it required disulphide bonding for biological activity. (2) It displaced PDGF from its receptors on fibroblasts (Fig. 2). (3) When added to an anti-PDGF antibody, supernatant from activated monocytes as well as the partially purified factor displaced ¹²⁵I-PDGF from the antibody in a similar pattern to PDGF (undiluted supernatant from activated

* Present addresses: University of Minnesota, Minneapolis, Minnesota 55455, USA (P.B.B.); Hôpital Louis Pradel, 69394 Cedex 3, Lyon, France (J.-F.M.); Medical University of South Carolina, Charleston, South Carolina 29425, USA (G.R.G.).

Fig. 1 Release of a mediator showing PDGF-like activity by activated human blood monocytes. *a*, Activated monocytes release a mediator possessing chemotactic activity for smooth muscle cells (SMC). *b*, Demonstration that the mediator with chemotactic activity for smooth muscle cells also exhibits PDGF-like growth activity for fibroblasts. The figure shows the number of fibroblasts (as % above the control of DMEM alone) with dilutions of the partially purified monocyte mediator (●) (starting concentration 50 μg protein per ml) or with dilutions of PDGF (○; starting concentration 25 ng ml^{-1}). Culture with the monocyte mediator alone, insulin alone or PDGF alone yielded no growth. All data points represent duplicates. Confirmation of the PDGF-like growth activity of the monocyte mediator was obtained by autoradiographic demonstration of ^3H -thymidine incorporation into nuclei of confluent BALB/c-3T3 (clone A-31; American Type Culture Collection CCL 163) in the presence of partially purified monocyte mediator and 50 $\mu\text{g ml}^{-1}$ insulin (not shown).

Methods. *a*, Monocytes were obtained by plating (plastic dish, 30 min, 37 °C) mononuclear cells isolated from blood by Hypaque-Ficoll centrifugation. Adherent cells (99% mononuclear cells, >87% monocytes) were cultured (4×10^6 cells ml^{-1} , 24 h, 37 °C) in Dulbecco's modified Eagle's medium (DMEM). In parallel cultures various stimuli were added: opsonized zymosan (zymosan A particles, Sigma; 10 particles per cell), immune complexes (human and rabbit anti-human albumin, Cappel; final protein concentration of 20 $\mu\text{g ml}^{-1}$), 1 LPS (*Escherichia coli* 0127; B8, Difco; 10 $\mu\text{g ml}^{-1}$). After culture (24 h, 37 °C), supernatants were collected and tested for chemotactic activity for SMC using a modified Boyden chamber³². SMC grown from explants of the medial layer of fetal bovine aortas³³ were suspended (4×10^5 cells ml^{-1}) in DMEM and placed in the upper wells; lower wells contained the supernatants to be tested. The filters (polycarbonate, 8 μm diameter pore size, Neuro Probe) were coated (72 h, 22 °C) with type V collagen obtained from human placental membranes. After incubation (4 h, 37 °C), the filters were removed, the cells were fixed and stained with Diff-Quik (American Scientific Products) and numbers of migrating SMC were determined³². Each sample was tested in duplicate. The results (mean $\pm$ s.d. for 3 experiments) represent the value for each condition minus the chemotactic activity in response to DMEM alone. None of the stimuli by themselves had chemotactic activity. The attraction of the SMC by monocyte supernatants was demonstrated by checkerboard analysis to be concentration-gradient dependent, that is, chemotactic. Purified PDGF (5 ng ml^{-1})³⁴⁻³⁷ was used as a positive control. For the monocyte supernatants, the data are presented as chemotactic units present in supernatant of 10^6 monocytes cultured for 24 h. *b*, Monocyte supernatant obtained by culture of blood monocytes with LPS was dialysed for 48 h against 10 mM NaCl, 35 mM Na citrate, pH 7.0, and mixed overnight with carboxymethyl-cellulose (CMC, CM 52, Whatman). The CMC was then packed in a column, washed and eluted with a linear gradient (10 mM–1 M NaCl). The SMC chemotactic activity eluted with the front of the protein peak at 0.35 M NaCl. All active fractions were pooled and applied on a blue-Sepharose column (Affi-Gel Blue; Bio-Rad) which was eluted with 1 M NaCl, 35 mM Na citrate, pH 7.0, v/v ethylene glycol/H₂O. In parallel with SMC chemotactic activity, the growth activity for fibroblasts in this eluate was evaluated by a complementation test¹¹ using diploid human lung fibroblasts (HFL-1, American Type Culture Collection CCL 153)³⁸. Non-cycling fibroblasts (culture in DMEM plus 0.4% calf serum for 4 days with an additional day in DMEM) were placed in DMEM with progressive dilutions of the blue-Sepharose eluate or PDGF in the presence of insulin (50 $\mu\text{g ml}^{-1}$; Collaborative Research). The fibroblasts were incubated for 3 days and then counted.

monocytes contained 10 ng ml^{-1} of the PDGF-like mediator; not shown).

The concept that activated monocytes release a PDGF-like mediator corresponds with the observation by Shimokado *et al.*¹⁴ that activated macrophages secrete a PDGF-like molecule. Furthermore, it is consistent with various observations concerning the role of monocytes and their progeny in certain physiological and pathological situations. First, the classical description of wound healing includes the recruitment of monocytes to the wound, followed by the local accumulation and proliferation of mesenchymal cells. Consistent with the view that monocytes are central to this process, Leibovich and Ross¹ observed marked suppression of wound healing in animals depleted of mononuclear phagocytes. Second, in human fibrotic disorders, the accumulation and proliferation of mesenchymal cells and the subsequent formation of scar are invariably preceded by the accumulation of mononuclear phagocytes^{4,5}. Third, animal models of atherosclerosis demonstrate the sub-endothelial accumulation of monocytes, followed by the recruitment of SMC and the development of the atherosclerotic plaque^{2,3}. Finally, *in vitro* studies have shown that activated monocytes release 'monocyte-derived growth factor'¹⁵, which possesses growth-competence activity for fibroblasts.

Since one of the PDGF chains (PDGF-2, B chain or peptide I) is encoded by the *c-sis* proto-oncogene (a gene on chromosome 22)^{8,9}, the fact that activated monocytes release a PDGF-like molecule suggests that monocyte activation is associated with the expression of *c-sis*. Consistent with this, we found that hybridization of monocyte poly(A)⁺ RNA with a ^{32}P -labelled probe containing exons 6 and 7 of *c-sis* revealed the presence of a 4.2-kilobase (kb) *c-sis* messenger RNA transcript in activated but not in resting monocytes (Fig. 3). In contrast, using a probe for the γ -actin gene, we found that both resting and activated monocytes express the γ -actin gene 2.0-kb mRNA transcript, consistent with the knowledge that the γ -actin gene is expressed in all cell types¹⁶.

The fact that human monocytes, when activated, express the

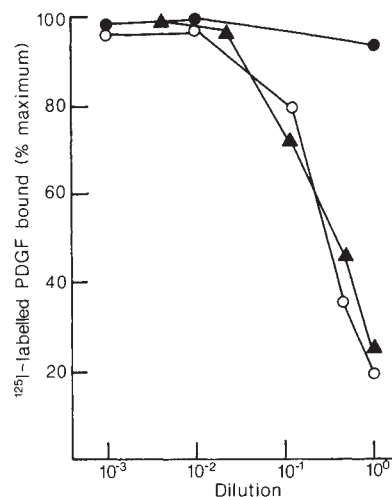
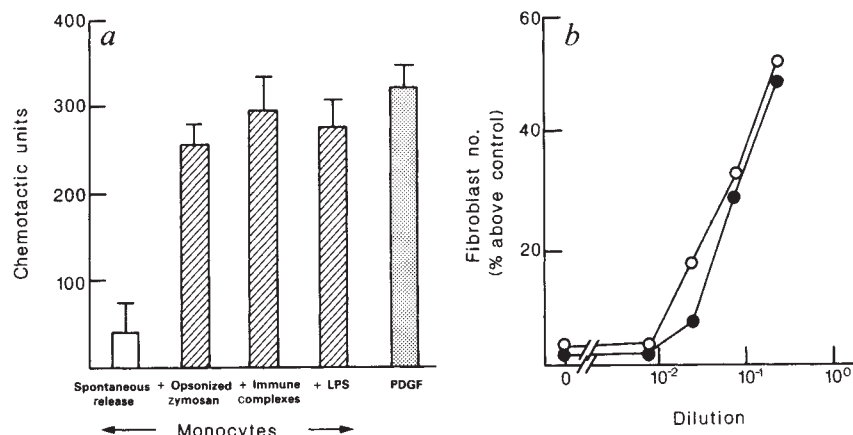


Fig. 2 Competition of the partially purified monocyte-derived factor with ^{125}I -labelled PDGF for binding to fibroblasts. Pure PDGF (1 μg) was labelled with ^{125}I using the chloramine-T procedure of Hunter *et al.*³⁹ and separated from free iodine by gel filtration on a PD-10 column (Pharmacia) previously equilibrated with 1 M acetic acid (titrated to pH 3.5 with NH_4OH) containing 1 mg ml^{-1} bovine serum albumin (BSA). The ^{125}I -labelled PDGF has a specific activity of 2×10^5 d.p.m. per ng. Receptor binding studies were performed as described by Heldin *et al.*⁴⁰. BALB/c-3T3 fibroblasts (clone A-31; American Type Culture Collection CCL 163) were cultured for 5 days in DMEM with 10% calf serum in 24-well Falcon dishes (Becton-Dickinson) at which time they exhibited density arrest of growth. The cells were washed twice with binding medium (2 mM CaCl_2 , 1 mM MgSO_4 , 1 mg ml^{-1} BSA in phosphate-buffered saline (PBS)) and incubated with 5 ng (10^5 d.p.m.) of ^{125}I -PDGF containing: (1) progressive dilutions of non-labelled PDGF (○, starting concentration 1 $\mu\text{g ml}^{-1}$); (2) partially purified monocyte-derived factor (CMC, followed by blue-Sepharose, see Fig. 1b legend) (▲, starting concentration 400 $\mu\text{g ml}^{-1}$); (3) fibroblast growth factor (●, starting concentration 1 $\mu\text{g ml}^{-1}$, Collaborative Research) as a control. After incubation (90 min, 22 °C) the cells were washed four times with washing media (2 mM CaCl_2 , 1 mM MgSO_4 , 1% calf serum in PBS) and lysed with lysing media (1% Triton X-100, 10% glycerol, 1 mg ml^{-1} BSA in 20 mM HEPES, pH 7.4). The bound ^{125}I -labelled PDGF activity was then quantified using a γ counter. All binding assays were done in duplicate; the data shown represent the mean values for three experiments.

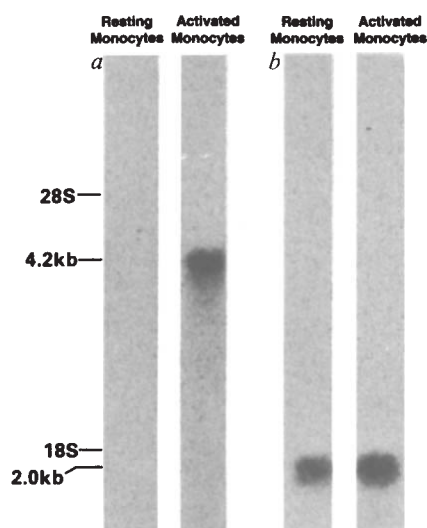


Fig. 3 Expression of the *c-sis* proto-oncogene in human monocytes. *a*, Presence of *c-sis* transcripts in activated monocytes; *b*, presence of γ -actin mRNA transcripts in both resting and activated monocytes.

Methods. Blood monocytes were cultured for 24 h with or without LPS (see Fig. 1a legend). After incubation, the cells were washed three times at 4°C in PBS, pH 7.4, collected with a rubber policeman and pelleted by centrifugation (2,000g, 5 min). The cell pellet was homogenized in guanidine hydrochloride and subjected to ultracentrifugation through a caesium chloride cushion⁴¹. Total RNA was enriched for poly(A)⁺ RNA by one cycle of oligo(dT)-cellulose affinity chromatography⁴². The poly(A)⁺ RNA (5 µg each lane) samples were subjected to electrophoresis in a 1.1% agarose formaldehyde gel⁴³ and transferred to a nitrocellulose filter. The filter was hybridized with probes radioactively labelled with ³²P by nick-translation⁴⁴. Hybridization and washing conditions were as described by Thomas⁴⁵. RNA sizes were determined by comparison with human (28S, 18S) ribosomal RNA. The *c-sis* probe was clone pL335, a subclone of λ -L33 containing exons 6 and 7 of the human *c-sis* locus¹⁷ kindly provided by E. Gelmann (NCI). The autoradiogram was exposed for 72 h. For *b*, the same filter as in *a* was used. After being allowed to undergo several half-lives of ³²P decay of the *c-sis* probe, the filter was hybridized to a human fibroblast cytoplasmic γ -actin cDNA (plasmid pHF γ A-1)⁴⁶ kindly provided by L. Keddes (Stanford University). The autoradiogram was exposed for 8 h.

c-sis proto-oncogene in parallel with the release of a molecule showing PDGF-like activity is of interest in view of the known relationship of *c-sis* to the *v-sis* homologous sequences in the simian sarcoma transforming retrovirus¹⁷, which induces NIH 3T3 fibroblasts and normal rat kidney cells to produce p28^{v-sis} (ref. 18), a protein of relative molecular mass 28,000 showing close homology to one of the PDGF chains¹⁹⁻²¹. Since PDGF is a growth factor that induces its target cells to enter the G₁ phase of the cell cycle¹¹, the finding that a transforming virus contains sequences encoding a protein similar to one of the PDGF chains, and that the normal human genome contains sequences with >90% homology with p28^{v-sis}, has important implications for the initial events leading to malignancy. Consistent with these observations, a PDGF-like molecule has been detected in the culture media of human sarcoma²² and glioma²³ and these cells express *c-sis* mRNA transcripts^{24,25}.

In addition to implications of the activation of *c-sis* for malignant growth, there is preliminary evidence that this proto-oncogene is involved in normal cell growth. In this context, cultured human and bovine endothelial cells express *c-sis*^{26,27} and release a PDGF-like molecule²⁸, while rat arterial SMC release a PDGF-like protein²⁹. Furthermore, using *in situ* hybridization, Goustin *et al.*³⁰ have recently demonstrated the presence of *c-sis* transcript in the cytotrophoblast of first-trimester human placenta in parallel with the release of a PDGF-like growth activity in the culture media. The present study extends these findings by demonstrating that *c-sis* proto-oncogene expression and release of a PDGF-like molecule occur in the activated human monocyte, a cell of major importance in scar formation and human fibrotic disorders as well as the early development of atherosclerotic plaque. These observations

support the theory that common mechanisms are involved in the 'controlled' proliferation of cells involved in normal growth, the 'semi-controlled' proliferation of cells involved in the chronic inflammatory disorders, and the 'uncontrolled' proliferation of malignant cells.

We thank E. Gelmann, G. Wilding, N. Davidson and C. Agnor for help in carrying out this project and la Fondation pour la Recherche Médicale (for Y.M.) and Ministère des Relations Extérieures, France (for J.-F.M.) for partial financial support. *Note added in proof:* Since submission of this manuscript, Shimokado *et al.*³¹ have also demonstrated that macrophages express *c-sis* and produce PDGF.

Received 25 July; accepted 18 October 1985.

- Leibovich, S. J. & Ross, R. *Am. J. Path.* **78**, 71-100 (1975).
- Gerrity, R. G. *Am. J. Path.* **103**, 181-190 (1981).
- Faggiotto, A., Ross, R. & Harker, L. *Arteriosclerosis* **4**, 323-340 (1984).
- Crystal, R. G., Bitterman, P. B., Rennard, S. I., Hance, A. J. & Keogh, B. A. *New Engl. J. Med.* **310**, 154-166; 235-244 (1984).
- Kent, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 3719-3722 (1976).
- Westermarck, B. *et al. in Growth and Maturation Factors* Vol. 1 (ed. Guroff, G.) 73-115 (Wiley, New York, 1983).
- Deuel, T. F. & Huang, J. S. *J. clin. Invest.* **74**, 669-676 (1984).
- Johnsson, A. *et al. EMBO J.* **3**, 921-928 (1984).
- Chiu, I. M. *et al. Cell* **37**, 123-129 (1984).
- Grotendorst, G. R., Pencev, D., Martin, G. R. & Sodek, J. *in Soft and Hard Tissue Repair* (eds Hunt, T. K., Heppenstall, R. B., Pines, E. & Rovee, D.) 20-40 (Praeger, New York, 1984).
- Pledger, W. J., Stiles, C. D., Antoniades, H. N. & Scher, C. D. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4481-4485 (1977).
- Bitterman, P. B., Rennard, S. I., Adelberg, S. & Crystal, R. G. *J. Cell Biol.* **97**, 1925-1932 (1983).
- Alitalo, K., Hovi, T. & Vaheri, A. *J. exp. Med.* **151**, 602-613 (1980).
- Shimokado, K., Raines, E. W., Madtes, D. K. & Ross, R. *J. Leukocyte Biol.* **37**, 742-743 (1985).
- Leibovich, S. J. & Ross, R. *Am. J. Path.* **84**, 501-514 (1976).
- Firtel, R. A. *Cell* **24**, 6-7 (1981).
- Dalla Favera, R., Gelmann, E. P., Gallo, R. C. & Wong-Staal, F. *Nature* **292**, 31-35 (1981).
- Devare, S. G., Reddy, E. P., Law, J. D., Robbins, K. C. & Aaronson, S. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 731-735 (1983).
- Doolittle, R. F. *Science* **221**, 275-277 (1983).
- Waterfield, M. D. *et al. Nature* **304**, 35-39 (1983).
- Robbins, K. C., Antoniades, H. N., Devare, S. G., Hunkapiller, M. W. & Aaronson, S. A. *Nature* **305**, 605-608 (1983).
- Heldin, C. H., Westermarck, B. & Wasteson, Å. *J. cell. Physiol.* **105**, 235-246 (1980).
- Nistér, M., Heldin, C. H., Wasteson, Å. & Westermarck, B. *Ann. N.Y. Acad. Sci.* **397**, 25-33 (1982).
- Eva, A. *et al. Nature* **295**, 116-119 (1982).
- Graves, D. T. *et al. Science* **226**, 972-974 (1984).
- Barrett, T. B., Gajdusek, C. M., Schwartz, S. M., McDougall, J. K. & Benditt, E. P. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6772-6774 (1984).
- Jaye, M. *et al. Science* **228**, 882-885 (1985).
- DiCorleto, P. E. & Bowen-Pope, D. F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1919-1923 (1983).
- Nilsson, J., Sjölund, M., Palmberg, L., Thyberg, J. & Heldin, C. H. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4418-4422 (1985).
- Goustin, A. S. *et al. Cell* **41**, 301-312 (1985).
- Shimokado, K. *et al. Cell* **43**, 277-286 (1985).
- Bleiberg, I., Harvey, A. K., Smale, G. & Grotendorst, G. R. *J. cell. Physiol.* **123**, 161-166 (1985).
- Ross, R. *J. Cell Biol.* **50**, 172-186 (1971).
- Antoniades, H. N., Scher, C. D. & Stiles, C. D. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1809-1813 (1979).
- Deuel, T. F. *et al. J. Biol. Chem.* **256**, 8896-8899 (1981).
- Heldin, C. H., Westermarck, B. & Wasteson, Å. *Biochem. J.* **193**, 907-913 (1981).
- Raines, E. W. & Ross, R. *J. Biol. Chem.* **257**, 5154-5160 (1982).
- Bitterman, P. B., Rennard, S. I., Hunninghake, G. W. & Crystal, R. G. *J. clin. Invest.* **70**, 806-822 (1982).
- Hunter, W. M. & Greenwood, F. C. *Nature* **194**, 495-496 (1962).
- Heldin, C. H., Westermarck, B. & Wasteson, Å. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3664-3668 (1981).
- Glisin, V., Crkvenjakov, R. & Byus, C. *Biochemistry* **13**, 2633-2637 (1974).
- Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408-1412 (1972).
- Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. *Biochemistry* **16**, 4743-4751 (1977).
- Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).
- Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5205 (1980).
- Gunning, P. *et al. Molec. cell. Biol.* **3**, 787-795 (1983).

Erratum

A closely linked genetic marker for cystic fibrosis

Ray White, Scott Woodward, Mark Leppert, Peter O'Connell, Yusuke Nakamura, Mark Hoff*, John Herbst†, Jean-Marc Lalouel, Michael Dean & George Vande Woude

Nature **318**, 382-384 (1985).

IN this letter, one of the authors' names was omitted (Y.N.). Yusuke Nakamura is at the Howard Hughes Medical Institute.

Mathematics

Analytical and Numerical Methods for Volterra Equations. By PETER LINZ. Wiley: 1985. Pp.227. ISBN 0-89871-198-3. £28.25.

Applied Linear Regression, 2nd Edition. By SANFORD WEISBERG. Wiley: 1985. Pp.324. Hbk ISBN 0-471-87957-6. £35.75.

The Beauty of Doing Mathematics: Three Public Dialogues. By SERGE LANG. Springer-Verlag: 1985. Pp.127. Pbk ISBN 3-540-96149-6. DM 66.

Computational Methods for Integral Equations. By L.M. DELVES and J.L. MOHAMED. Cambridge University Press: 1985. Pp.376. Hbk ISBN 0-521-26629-7. £35, \$69.50.

Dynamical Problems in Soliton Systems. S. TAKE-NO (ed.). Springer-Verlag: 1985. Pp.281. ISBN 3-540-15372-1/0-387-15372-1. DM 89.

The Elements of Graphic Data. By WILLIAM S. CLEVELAND. Wadsworth: 1985. Pp.323. Pbk ISBN 0-534-03730-5. Hbk \$27.95; pbk £18.95.

Encyclopedia of Statistical Sciences. Vol. 6 Multivariate Analysis — Plackett and Burman Designs. S. KOTZ, N.L. JOHNSON AND C.B. READ (eds). Wiley: 1985. Pp.758. ISBN 0-471-05553-0. £74.

Parallel Sorting Algorithms. By SELIM G. AKL. Academic: 1985. Pp.229. Hbk ISBN 0-12-047680-0. £43.50, \$49.50.

Recursive Estimation and Control for Stochastic Systems. By HANS-FU CHEN. Wiley: 1985. Pp.378. ISBN 0-471-81566-7. £46.20.

Solving Equations with Physical Understanding. By J.R. ACTON and P.T. SQUIRE. Adam Hilger: 1985. Pp.219. Hbk ISBN 0-85274-757-8; pbk ISBN 0-85274-789-5. Hbk £24, \$36; pbk £12.95, \$19.

Statcalc. By T.J. BROWNE and R.B.G. WILLIAMS. Macmillan: 1985. BBC Model B 40 Track. ISBN 0-333-39598-0. £26.50.

Astronomy

Astronomy From Space. G.G. FAZIO, J.A.M. BLEEKER, P.A.J. DE KORTE and J.J. CALDWELL (eds). Pergamon Press: 1985. Pp.212. Pbk ISBN 0-08-033192-0. Pbk £31, \$49.50.

Astronomy with a Small Telescope. By JAMES MUIRDEN. George Philip, 12-14 Long Acre, London WC2E, UK: 1985. Pp.224. Hbk ISBN 0-540-01088-X. £9.95.

The Case for Mars II. Vol. 62, Science and Technology Series. CHRISTOPHER P. MCKAY (ed.). Published for the American Astronautical Society by Univelt, Inc., P.O. Box 28130, San Diego, California, USA: 1985. Pbk ISBN 0-87703-220-3. Np.

Europe/United States Space Activities, 23rd Goddard Memorial Symposium, 19th European Space Symposium. PETER M. BAINUM and FRIEDRICH VON BUN (eds). Published for the American Astronautical Society by Univelt, Inc., P.O. Box 28130, San Diego, California, USA: 1985. Np.

Guidance and Control 1985. Vol. 57 Advances in the Astronautical Sciences. ROBERT D. CULP, EDWARD J. BAUMAN and CHARLES A. CULLIAN (eds). American Astronautical Society: 1985. Pp.601. ISBN Hbk 0-87703-211-4; pbk ISBN 0-87703-212-2. Hbk \$65; pbk \$50.

Space Debris, Asteroids and Satellite Orbits. D.J. KESSLER, E. GRUN and L. SEHNAL (eds). Pergamon: 1985. Pp.229. Pbk ISBN 0-08-033189-0. Pbk £31, \$49.50.

A Spacefaring People: Perspectives on Early Spaceflight. By ALEX ROLAND. Scientific and Technical Information Branch, National Aeronautics and Space Administration, Washington, USA: 1985. Pp.156. Pbk. Np.

Spherical Astronomy. By ROBIN M. GREEN. Cambridge University Press: 1985. Pp.520. Hbk ISBN 0-521-23988-5; pbk ISBN 0-521-31779-7. Hbk £40, \$69.50; pbk £15, \$27.95.

The Universe. By IAIN NICOLSON and PATRICK MOORE. Collins: 1985. Pp.248. Hbk ISBN 0-00-217397-2. £15.

Vistas in Astronomy: An International Review Journal. Vol. 28, Parts 1,2. P. BEER, A.J. MEADOWS and A.E. ROY (eds). Pergamon: 1985. Pp.404. £50, \$81.

Physics

Clustering Aspects of Nuclear Structure. J.S. LILLEY and M.A. NAGARAJAN (eds). Reidel, The Netherlands: 1985. Pp.420. Hbk ISBN 90-277-2002-9. Dfl. 160, £44.50, \$54.

Electronic Properties of Polymers and Related Compounds. H. KUZMANY, M. MEHRING and S. ROTH (eds). Springer-Verlag: 1985. Pp.354. ISBN 3-540-15722-0/0-387-15722-0. DM 89.

Groups Theory in Physics. By WU-KI TUNG. Wiley: 1985. Pp.344. Pbk ISBN 9971-966-57-3. £17.45.

Highlights of Condensed-Matter Theory. F. BASANI, F.G. FUMI and M.I. TOSI (eds). North-Holland Physics Publishing, P.O. Box 103, 1000 AC Amsterdam, The Netherlands: 1985. Pp.916. Hbk ISBN 0-444-86976-X. Dfl. 340.

High-Power Laser Radiation in Atmospheric Aerosols. By V.E. ZUEV, A.A. ZEMLYANOV, YU. D. KOPYTIN and A.V. KUZIKOVSKII. Reidel: 1985. Pp.291. Hbk ISBN 90-277-1736-2. Np.

High-Resolution Spectroscopy of Transient Molecules. By EIZI HIROTA. Springer-Verlag: 1985. Pp.233. ISBN 3-540-15302-0. DM 125.

Large-Scale Computations in Fluid Mechanics. BJORN E. ENGUIST, STANLEY OSHER and RICHARD C.J. SOMERVILLE (eds). American Mathematical Society: 1985. Pp.409. ISBN 0-8218-1122-3. \$110.

Microcomputer Quantum Mechanics, 2nd Edn. By J.P. KILLINGBECK. Adam Hilger: 1985. Pp.187. ISBN 0-85274-803-5. £12.95, \$20.

Modern Electronics and Integrated Circuits. By B.J. STANIER. Adam Hilger: 1985. Pp.152. Hbk ISBN 0-85274-550-8; pbk ISBN 0-85274-552-4. Hbk £20, \$32; pbk £10, \$15.

Optical Bistability: Controlling Light with Light. By HYATT M. GIBBS. Academic: 1985. Pp.471. Hbk ISBN 0-12-281940-3. £47.50, \$54.50.

Physics and Contemporary Needs, Vol. 7. M.N. QAZI (ed.). Wiley: 1985. Pp.296. Hbk ISBN 9971-966-83-2. £42.30.

Physics of Dense Matter. By Y.C. LEUNG. Wiley: 1985. Pp.268. ISBN 9971-978-10-5. £38.10.

Plasma Physics. By R.A. CAIRNS. Blackie: 1985. Pp.193. Hbk ISBN 0-216-91779-4; pbk ISBN 0-216-91783-2. Hbk £19.95; pbk £9.95.

Plasma Polymerization. By H. YASUDA. Academic: 1985. Pp.432. ISBN 0-12-768760-2. \$62, £62.

Polarized Electrons at Surfaces. By J. KIRSCHNER. Springer-Verlag: 1985. Pp.158. ISBN 3-540-15003-X/0-387-15003-X. DM 69.

Polarized Light in Nature. By G.P. KÖNNEN. Cambridge University Press: 1985. Pp.172. ISBN 0-521-25862-6. £19.50, \$32.50.

Problems in Perturbation. By ALI HASAN NAYFEH. Wiley: 1985. Pp.556. ISBN 0-471-82292-2. £40.85.

Pseudopotential Theory of Atoms and Molecules. By LEVENTE SZASZ. Wiley: 1985. Pp.309. Hbk ISBN 0-471-82417-8. £56.20.

Response and Stability. An Introduction to the Physical Theory. By A.B. PIPPARD. Cambridge University Press: 1985. Pp.228. Hbk ISBN 0-521-26673-4; pbk ISBN 0-521-31994-3. Hbk \$49.50, £27.50. Pbk \$17.95, £9.95.

Solar Radiophysics. D.J. McLEAN and N.R. LABRUM. Cambridge University Press: 1985. Pp.516. ISBN 0-521-25409-4. £35, \$59.50.

Solid State Physics, 2nd Edn. By J.S. BLACKMORE. Cambridge University Press: 1985. Pp.506. Hbk ISBN 0-521-30932-8; pbk ISBN 0-521-31391-0. Hbk £27.95, \$40.00; pbk £9.95.

A Survey of the Spherical Space Form Problem. By J.F. DAVIS and R.J. MILGRAM. Harwood: 1985. Pp.283. Pbk ISBN 3-7186-0250-4. Pbk \$19.

The Theory of Constancy Through Relativity by Means of Light Dynamics. By SHRI RAM SAGAR. Shri Ram Sagar, Balajipura, Amalner-425 401, Dist-Jalgaon (M.S.), India: 1985. Pp.125. Rs 30.

Thermal Conductivity 18. T. ASHWORTH and DAVID R. SMITH (eds). Plenum: 1985. Pp.764. ISBN 0-306-41918-1. Np.

Underwater Acoustics: A Linear Systems Theory Approach. By L.J. ZIOMEK. Academic: 1985. Pp.290. ISBN 0-12-781720-4. \$45, £45.

VLSI Electronics: Microstructure Science. NORMAN G. EINSBRUCH and ROBERT S. BAUER (eds). Academic: 1985. Pp.383. ISBN 0-12-234110-4. \$65, £65.

White Light: Optical Signal Processing. By FRANCIS T.S. YU. Wiley: 1985. Pp.316. Hbk ISBN 0-471-80954-3. £46.00.

Chemistry

Catalysis and Surface Science. HEINZ HEINEMANN and GABOR A. SOMORJAI (eds). Marcel Dekker: 1985. Pp.456. ISBN 0-8247-7328-4. \$75.

The Chemistry of the Metal-Carbon Bond. Vol. 3 Carbon-Carbon Bond Formation Using Organometallic Compounds. FRANK R. HARTLEY and SAUL PATAI (eds). Wiley: 1985. Pp.489. ISBN 0-471-90557-7. £98.

Formaldehyde: Analytical Chemistry and Toxicology. VICTOR TUROSKI (ed.). Advances in Chemistry Series, American Chemical Society: 1985. Pp.393. Hbk ISBN 0-8412-0903-0. (US and Canada) \$89.95, (Export) \$107.95.

Gas-Phase Chemiluminescence and Chemi-Ionization. ARTHUR FONTIJN (ed.). North-Holland: 1985. Pp.372. Hbk ISBN 0-444-86950-6. Dfl.110.

General Chemistry: Principles and Modern Applications 4th Edn. By RALPH H. PETRUCCI. Collier Macmillan: 1985. Pp.131. Pbk ISBN 0-02-946550-8. Pbk £17.95.

Higher Excited States of Polyatomic Molecules, Vol. III. By MELVIN B. ROBIN. Academic: 1985. Pp.465. ISBN 0-12-589903-3. \$49.50, £49.50.

Introducing Organic Chemistry, 3rd Edn. By ANDREW STREITWIESSER and CLAYTON H. HEATHCOCK. Collier Macmillan: 1985. Pp.1197. Pbk ISBN 0-02-946720-9. Pbk £17.95.

Mass Spectrometry of Heterocyclic Compounds, 2nd Edn. By Q.N. PORTER. Wiley: 1985. Pp.966. ISBN 0-471-09901-5. £289.15.

Mechanisms of Inorganic and Organometallic Reactions. M.V. TWIGG (ed.). Plenum: 1985. Pp.519. Hbk ISBN 0-306-41960-2. Np.

Methods for the Oxidation of Organic Compounds; Alkanes, Akenes, Alkynes, and Arenes. By ALAN H. HAINES. Academic: 1985. Pp.388. Hbk ISBN 0-12-315501-0. £75, \$83.

Organic Photochemistry. A. PADWA (ed.). Marcel Dekker: 1985. Pp.504. ISBN 0-8247-7421-3. \$89.75.

Oxidative Stress. HELMUT SIES (ed.). Academic: 1985. Pp.507. ISBN 0-12-642760-7. \$79, £72.

Polyimides: Synthesis, Characterization, and Applications. K.L. MITTAL (ed.). Plenum: 1984. Pp.1182. ISBN 0-306-41673-5. \$89.50.

Process Systems Engineering PSE '85: The Use of Computers in Chemical Engineering. INSTITUTION OF CHEMICAL ENGINEERS. Pergamon: 1985. Pp.684. ISBN 0-08-031417-1. £53, \$63.

The Proton: Applications to Organic Chemistry. By ROSS STEWART. Academic: 1985. Hbk ISBN 0-12-670370-1. \$65, £56.50.

The Structure of Volatile Sulphur Compounds. By ISTVÁN HARGITAI. Reidel: 1985. Pp.316. ISBN 90-277-1395-2. \$49, £34.50.

Synthetic Polymeric Membranes: A Structure Perspective, 2nd Edn. By ROBERT E. KESTING. Wiley: 1985. Pp.348. Hbk ISBN 0-471-80717-6. £55.75.

Technology

Fiber Optics: Technology and Applications. By STEWART D. PERSONICK. Plenum: 1985. Pp.257. Hbk ISBN 0-306-42079-1. Np.

Powtech '85: Particle Technology. INSTITUTION OF CHEMICAL ENGINEERS. Pergamon: 1985. Pp.294. ISBN 0-08-031443-0. £20, \$24.

The Social Shaping of Technology. DONALD MACKENZIE and JUDY WAJCMAN (eds). Open University Press: 1985. Pp.327. Pbk ISBN 0-335-15026-8. Pbk. Np.

Solvent Extraction and Ion Exchange in the Nuclear Fuel Cycle. D.H. LOGSDAIL and A.J. MILLS (eds). Wiley: 1985. Pp.223. ISBN 0-85312-12-842-1. £28.50.

Total Pressure Measurements in Vacuum Technology. By A. BERMAN. Academic:1985. Pp.396. Hbk ISBN 0-12-092440-4. £52, \$59.50.

Computer Science

Algorithmically Specialized Parallel Computers. LAWRENCE SNYDER *et al.* (eds). Academic: 1985. Pp.252. ISBN 0-12-654130-2. \$26.50, £24.

The Computer Culture: A Symposium to Explore the Computer's Impact on Society. DENIS P. DONNELLY (ed.). Fairleigh Dickinson University Press, Cranbury, New Jersey, USA: 1985. Pp.176. Hbk ISBN 0-8386-3220-3. £24.50.

Computer Speech Processing. FRANK FALLSIDE and WILLIAM A. WOODS (eds). Prentice-Hall: 1985. Pp.506. ISBN 0-13-163841-6. £39.95.

Fortran Optimization. By M. METCALF. Academic: 1985. Pp.251. ISBN 0-12-492482-4. \$26, £20.

Foundations of Programming. By JACQUES ARSAC. Academic: 1985. Pp.265. ISBN 0-12-064460-6. \$29.50, £23.

Getting Computers to Talk Like You and Me. By RACHEL REICHMAN. MIT Press: 1985. Pp.221. ISBN 0-262-18118-5. \$20.

An Introduction to Linear Programming, 2nd Edn. By G.R. WALSH. Wiley: 1985. Pp.240. Pbk ISBN 0-471-90719-7. Pbk £13.95.

Prolog for Programmers. By FELIKS KLUZNIAK and STANISLAW SZPAKOWICZ. Academic: 1985. Pp.400. ISBN 0-12-416520-6. \$47.50.

Stiff Computation. R.C. AIKEN (ed.). Oxford University Press: 1985. Pp.462. ISBN 0-19-503453-8. £72.

Earth Sciences

Dynamic Meteorology. By S. PANCHEV. D. Reidel: 1985. Pp.360. Hbk ISBN 90-277-1744-3. Dfl 200, £50.95, \$74.00.

The Earth's Surface Studied from Space. S.G. UNGAR (ed.). Pergamon: 1985. Pp.117. Pbk ISBN 0-08-033194-7. Pbk £31, \$49.50.

Explanatory Notes for the Geodynamic Map of the Circum Pacific Region. By GEORGE W. MOORE. American Association of Petroleum Geologists, PO Box 979, Tulsa, OK 74101: 1985. \$15.

Geomorphology and Soils. K.S. RICHARDS, R.R. ARNETT and S. ELLIS (eds). George Allen & Unwin: 1985. Pp.441. ISBN 0-04-551093-8. £35, \$60.

Groundwater Contamination. Studies in Geophysics. J. BREDEHOEFT *et al.* (eds). National Academy Press: 1984. Pp.179. Pbk ISBN 0-309-03441-8. Np.

Journal of Atmospheric Chemistry, Vol. 3, No.1, June 1985. Scientific Application of Baseline Observations of Atmospheric Composition (SABOAC), Part 1. PAUL J. CRUTZEN and DIETER H. EHHALT (eds). Reidel, Holland and USA: 1985. Pp.201. Pbk ISBN 0167-7764. Dfl.100, \$38.00.

Migmatites. J.R. ASHWORTH (ed.). Blackie: 1985. Pp.302. ISBN 0-412-00841-6. £27.50.

The Weather Book. By RALPH HARDY, PETER WRIGHT, JOHN GRIBBON and JOHN KINGTON. Mermaid Books: 1985. Pp.224. Pbk ISBN 0-7181-2639-4. £8.95.

Environmental Sciences

Chemical Water and Wastewater Treatment. A. GROHMANN, H.H. HAHN and R. KLUTE (eds). Gustav Fischer Verlag: 1985. Pp.310. ISBN 3-437-30503-4. Np.

Estuarine Management and Quality Assessment. J.G. WILSON and W. HALCROW (eds). Plenum: 1985. Pp.225. ISBN 0-306-41911-4. Np.

Ground Water Quality. C.H. WARD and P.L. McCARTY (eds). Wiley: 1985. Pp.545. ISBN 0-471-81597-7. £66.45.

Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Aromatic Amines and 4-Nitrophenyl. M. CASTEGNARO *et al.* (eds). Oxford University Press: 1985. Pp.85. Pbk ISBN 0-19-723064-4. Pbk £6.95.

Occupational Hazards of Pesticide Use. G.J. TURNBULL (ed.). Taylor & Francis: 1985. Pp.184. ISBN 0-85066-325-3. £19.

Troubled Skies, Troubled Waters: The Story of Acid Rain. By JON R. LUOMA. Penguin: 1985. Pp.178. Pbk ISBN 0-1400-8094-5. Pbk \$5.95.

Wastes in the Ocean, Vol. 4. Energy Wastes in the Ocean. IVER W. DUEDALL, DANA R. KESTER, P. KILHO PARK and BOSTWICK H. KETCHUM (eds). Wiley: 1985. Pp.818. Hbk ISBN 0-471-89332-3. £97.20.

Biological Sciences

Action Systems. An Introduction to the Analysis of Complex Behaviour. By DAVID D. CLARKE and JILL CROSSLAND. Methuen: 1985. Pp.157. Pbk ISBN 0-416-36130-7. £14.95.

The Alkaloids. A. BROSSI (ed.). Academic: 1985. Pp.357. ISBN 0-12-469524-8. \$85, £85.

Anales del Jardín Botánico de Madrid (Anales del Instituto Botánico, Tomo 41-II.) A.J. CAVANILLES. 2 Plaza de Murillo, 28014 Madrid, Spain: 1985. Pp.483. Pbk ISBN 0211-1322. Np.

Animal Thought. By STEPHEN WALKER. Routledge & Kegan Paul: 1985. Pp.437. Pbk ISBN 0-7102-0707-7. £6.95.

Annals of Child Development, Vol.2. GROVER J. WHITEHURST (ed.). JAI Press, 36 Sherwood Place, Greenwich, Connecticut, USA: 1985. Pp.297. Hbk ISBN 0-89232-546-1. Np.

The Aquarium Fish Survival Manual: A Comprehensive Guide to Keeping Freshwater and Marine Fish. By BRIAN WARD. Macdonald & Co.: 1985. Pp.176. Hbk ISBN 0-356-10792-2. £11.95.

Atherosclerosis. Annals of the New York Academy of Sciences, Vol. 454. K.T. LEE (ed.). The New York Academy of Sciences: 1985. Hbk ISBN 0-89766-303-9; pbk ISBN 0-89766-304-7. Np.

Atlas of Human Anatomy. By J.A. GOSLING, P.F. HARRIS, J.R. HUMPHERSON, I. WHITMORE and P.L.T. WILLAN. Gower Medical Publishing: 1985. Hbk ISBN 0-443-02913-X. £19.50.

Avian Biology, Vol. VIII. DONALD S. FARNER, JAMES R. KING and KENNETH C. PARKES (eds). Academic: 1985. Pp.256. ISBN 0-12-249408-3. \$49.50, £43.50.

Bacterial Adhesion: Mechanisms and Physiological Significance. DWAYNE C. SAVAGE and MADILYN FLETCHER (eds). Plenum: 1985. Pp.476. ISBN 0-306-41948-3. Np.

Bee Orchids. By STEPHEN BLACKMORE. Shire Natural History: 1985. Pp.24. Pbk ISBN 0-85263-745-4. Pbk £1.25.

Biochemistry of Dioxygen. By LLOYD L. ING-RAHAM and DAMON L. MEYER. Plenum: 1985. Pp.287. Hbk ISBN 0-306-41948-3. Np.

Biochemistry and Function of Vacuolar Adenosine-Triphosphatase in Fungi and Plants. BERNARD P. MARIN (ed.). Springer-Verlag: 1985. Pp.259. ISBN 3-540-15267-9/0-387-15267-9. DM 84.

Biology of Acanthocephala. D. CROMPTON and B. NICKOL (eds). Cambridge University Press: 1985. Pp.519. Hbk ISBN 0-521-24674-1. £60, \$89.50.

Biology of the Reptilia. Vol. 15. CARL GANS and FRANK BILLET (eds). Wiley: 1985. Pp.731. Hbk ISBN 0-471-81204-8. £81.80.

Biophysical Control of Microfibril Orientation in Plant Cell Walls: Aquatic and Terrestrial Plants including Trees. By J.D. BOYD. Dr W Junk: 1985. Pp.200. ISBN 90-247-3101-1. \$42, £30.50.

Biotin. KRISHNAMURTI DAKSHINAMURTI and HEMMIGE N. BHAGAVAN (eds). New York Academy of Sciences: 1985. Pp.441. Pbk ISBN 0-89766-289-X. Np.

British Warblers. By ERIC SIMMS. Collins: 1985. Pp.432. Pbk ISBN 0-00-219404-X. Np.

Buttercups. By STEPHEN BLACKMORE. Shire Natural History. Cromwell House, Church Street, Princes Risborough, Bucks HP17 9AJ: 1985. Pp.24. Pbk ISBN 0-85263-763-2. Pbk £1.25.

Cellular and Molecular Aspects of Aging: The Red Cell as a Model. JOHN W. EATON, DIANE K. KONZEN and JAMES G. WHITE (eds). Alan R. Liss: 1985. Pp.464. Hbk ISBN 0-8451-5045-6. £52.

Cellular and Molecular Biology of Lymphokines. CLEMENS SORG and ANNELIESE SCHIMPL (eds). Academic: 1985. Pp.845. Hbk ISBN 0-12-656160-5. £60, \$69.

Central and Peripheral Mechanisms of Colour Vision. D. OTTOSON and SEMIR ZEKI (eds). Macmillan: 1985. Pp.239. Hbk ISBN 0-333-39321-X. £60.

The Cereal Rusts: Vol II, Diseases, Distribution Epidemiology and Control. ALAN P. ROELFS and WILLIAM R. BUSHNELL (eds). Academic: 1985. Pp.606. Hbk ISBN 0-12-148402-5. £64.50, \$74.50.

Cerebral Cortex, Vol. 3: Visual Cortex. ALAN PETERS and EDWARD G. JONES (eds). Plenum: 1985. Pp.424. Hbk ISBN 0-306-42025-2. Np.

Cerebral Lateralization in Nonhuman Species. STANLEY D. GLICK (ed.). Academic: 1985. Pp.288. ISBN 0-12-286480. \$49.50, £49.50.

Chemically Mediated Interactions Between Plants and Other Organisms. GILLIAM A. COOPER-DRIVER, TONY SWAIN and ERIC E. CONN (eds). Plenum: 1985. Pp.246. ISBN 0-306-42006-6. Np.

The Chemistry and Biology of Isoquinoline Alkaloids. J.D. PHILLIPSON, M.F. ROBERTS and M.H. ZENK (eds). Springer-Verlag: 1985. Pp.304. ISBN 3-540-13980-X/0-387-13980-X. DM 108.

Communication and Noncommunication by Cephalopods. By MARTIN MOYNIHAM. Indiana University Press: 1985. Pp.141. ISBN 0-253-31382-1. \$32.50.

Contemporary Nephrology, Vol. 3. SAULO KLAHR and SHAUL G. MASSRY (eds). Plenum: 1985. Pp.768. Hbk ISBN 0-306-41984-X. Np.

Continuous Arteriovenous Hemofiltration (CAVH). H.G. SIEBERTH and H. MANN (eds). Karger: 1985. Pp.247. ISBN 3-8055-4077-9. DM 78, \$27.75.

Current Topics in Cellular Regulation. BERNARD L. HORECKER and EARL R. STADTMAN (eds). Academic: 1985. Pp.183. ISBN 0-12-152825-1. \$45, £45.

Earthworms. By R.W. SIMS and B.M. GERARD. The Linnean Society/E.J. Brill, Plantijnstraat 2, 2321 JC Leiden, Belgium: 1985. Pp.171. Pbk ISBN 90-04-07582-8. £12.50.

Ecological Interactions in Soil: Plants, Microbes and Animals. A.H. FITTER *et al.* (eds). Blackwell Scientific: 1985. Pp.451. ISBN 0-632-01386-9. £34.50.

Ecology and Genetics of Host-Parasite Interactions. Papers presented at an International Symposium organized by the Linnean Society of London and the British Society for Parasitology. Linnean Society Symposium Series No.11. D. ROLLINSON and R.M. ANDERSON (eds). Published for the Linnean Society of London by the Academic Press: 1985. Pp.266. Hbk ISBN 0-12-593690-7. £35, \$45.

The Encyclopaedia of Underwater Life. Dr KEITH BANISTER and Dr ANDREW CAMPBELL (eds). George Allen and Unwin: 1985. Pp.287. Hbk ISBN 0-04-500035-2. £25.

The Enzymes of Biological Membranes, 2nd Edn. ANTHONY N. MARTONOSI (ed.). Plenum: 1985. Pp.586. ISBN 0-306-41454-6. Np.

The Evolution of Genome Size. T. CAVALIER-SMITH (ed.). Wiley: 1985. Pp.523. Hbk ISBN 0-471-10272-5. £38.50.

Flora of Kuwait. By HAZIN S. DAOUD. KPI, 14 Leicester Square, London WC2H 7PH, England: 1985. Pp.224. ISBN 0-7103-0075-1. £45.

Frontiers in Histamine Research: A Tribute to Heinz Schild. C.R. GANELLIN and J.C. SCHWARTZ (eds). Pergamon: 1985. Pp.442. ISBN 0-08-031989-0. £50, \$80.

Functions of the Brain. CLIVE WARWICK COEN (ed.). Oxford University Press: 1985. Pp.209. Hbk ISBN 0-19-857212-3. £15.

Fungi. By P. FOREY. Shire Natural History: 1985. Pp.24. Pbk ISBN 0-85263-746-2. Pbk £1.25.

Gene Expression During Normal and Malignant Differentiation. LEIF C. ANDERSSON, C.G. GAHM-BERG and P. EKBLOM (eds). Academic: 1985. Pp.257. ISBN 0-12-059490-0. \$30, £26.50.

God's Acres: The Flowers and Animals of the Parish Churchyard. By FRANCESCA GREENOAK. Orbis Publishing, 20-22 Bedfordbury, London WC2N 4BT, UK: 1985. Pp.192. ISBN 0-85613-800-2. £12.95.